Recent Advances in Metal Carcinogenesis*†

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ABSTRACT

Recent advances in metal carcinogenesis are comprehensively reviewed, including (a) epidemiological and clinical aspects, (b) carcinogenesis bioassays, (c) bacterial mutagenesis, (d) mammalian cell mutagenesis, (e) chromosomal damage, (f) mammalian cell transformation, (g) microsomal metabolism, (h) DNA strandbreaks and crosslinks, (i) DNA polymerase infidelity, (j) RNA strand initiation, and (k) helical transition of B-DNA to Z-DNA. Based upon these observations, several hypotheses are proposed for the molecular pathogenesis of carcinogenesis by metal compounds. These hypotheses are amenable to experimental test by existing techniques of molecular biology.

Introduction

This article summarizes the remarkable advances in understanding of metal carcinogenesis that have occurred during the past four years. The discussion is limited to research published from 1979 to July 1983. For an overview of earlier literature, readers should consult general reviews of metal carcinogenesis82,84,85,160-163 and papers on As,56,75,96,136 Be,75,91 Cd,40,74,138 Cr,62,63,66,75,97,99,125 Ni,57,74,95,140,164-166 and Pb.75,115 For orientation, background information on metal carcinogenesis in animals are summarized in table II.

Epidemiology and Clinical Aspects

Arsenic

Recent studies (table III) confirmed previous reports that copper smelter workers, who are exposed to inhalation of AsO3 dust, have excess risk of lung cancer mortality. Higgins et al67 showed that lung cancer incidence is correlated with atmospheric As-levels. Enterline and Marsh46,47 noted that lung cancers can develop within ten years after initial exposure, and that the effects of arsenic exposure tend to disappear with time, suggesting that arsenic may act as a cancer promoter rather than as an initiator. Consistent with this hypothesis, Brown and Chu17 reported that lung cancer mortality of arsenic-exposed...
TABLE I
METAL EXPOSURES ASSOCIATED WITH HUMAN CANCERS (160-163)a

<table>
<thead>
<tr>
<th>Metals</th>
<th>Exposures</th>
<th>Routes</th>
<th>Malignant Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>As</td>
<td>Drinking water; Fowler's solution</td>
<td>Oral</td>
<td>Skin carcinomas, liver angiosarcomas</td>
</tr>
<tr>
<td></td>
<td>Smelting; pesticide production &amp; use</td>
<td>Inhalation</td>
<td>Lung and skin carcinomas, lymphomas, leukemias</td>
</tr>
<tr>
<td>Cr</td>
<td>Smelting; production of chromates</td>
<td>Inhalation</td>
<td>Lung carcinomas</td>
</tr>
<tr>
<td>Ni</td>
<td>Refining</td>
<td>Inhalation</td>
<td>Lung and sinonasal carcinomas</td>
</tr>
</tbody>
</table>

a Limited evidence also suggests that exposures to certain Be, Cd, and Fe compounds lead to increased risks of specific types of cancers (74,75,161).

TABLE II
CARCINOGENICITY OF METAL COMPOUNDS IN EXPERIMENTAL ANIMALSa,b

<table>
<thead>
<tr>
<th>Metals</th>
<th>Typical Compounds</th>
<th>Routesc</th>
<th>Principal Neoplasms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Be</td>
<td>BeO, BeHPO4</td>
<td>iv, inh, ios</td>
<td>lung carcinomas, bone sarcomas</td>
</tr>
<tr>
<td>Cd</td>
<td>Cd, CdS, CdCl₂</td>
<td>im, sc, its</td>
<td>local sarcomas, gonadal teratomas</td>
</tr>
<tr>
<td>Co</td>
<td>Co, CoS, CoCl₂</td>
<td>im, sc, ios</td>
<td>local sarcomas</td>
</tr>
<tr>
<td>Cr</td>
<td>CaCrO₄, PbCrO₄</td>
<td>inh, im, sc</td>
<td>lung carcinomas, local sarcomas</td>
</tr>
<tr>
<td>Fe</td>
<td>Fe-carbohydrates</td>
<td>im, ip, sc</td>
<td>local sarcomas</td>
</tr>
<tr>
<td>Ni</td>
<td>Ni₃S₂, Ni(CO)₄</td>
<td>im, inh, iv</td>
<td>lung carcinomas, local sarcomas</td>
</tr>
<tr>
<td>Pb</td>
<td>Pb(C₂H₃O₂)₂</td>
<td>po, ip, sc</td>
<td>renal carcinomas</td>
</tr>
<tr>
<td>Pt</td>
<td>cis-Pt drugs</td>
<td>sc, ip</td>
<td>local sarcomas, lung adenomas</td>
</tr>
</tbody>
</table>

a In addition, Al-dextran, Mn-acetylacetone, and Ti(C₅H₅)₂ induce local sarcomas after im or sc injection; CuCl₂ and ZnSO₄ induce gonadal teratomas after intratesticular injection.

b For bibliographic citations and tabulations of the various species in which the metal compounds have induced tumors, readers should consult previous reviews (74,75,160-163).

c Im = intramuscular, inh = inhalation, ios = intraosseous, ip = intraperitoneal, ir = intrarenal, its = intratesticular, iv = intravenous, po = oral, sc = subcutaneous.
### Table III

**Lung Cancer Mortality in Arsenic-Exposed Workers**

<table>
<thead>
<tr>
<th>Authors &amp; Country</th>
<th>Worker Population</th>
<th>Year of First Employment</th>
<th>Lung Cancer Mortality Obs(0)</th>
<th>Exp(E)</th>
<th>(O/E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lubin et al. (106) USA</td>
<td>Copper smelter</td>
<td>1938-56</td>
<td>139</td>
<td>83.9</td>
<td>1.66&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Higgins et al. (67) USA</td>
<td>Copper smelter&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1938-56</td>
<td>High As</td>
<td>6</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Med. As</td>
<td>5</td>
<td>2.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Low As</td>
<td>3</td>
<td>1.77</td>
</tr>
<tr>
<td>Enterline &amp; Marsh (46,47) USA</td>
<td>Copper smelter</td>
<td>1940-64</td>
<td>100</td>
<td>51.30</td>
<td>1.95&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Sub-group of the population studied by Lubin et al. (106).

<sup>b</sup> P < 0.01.

Workers increased with age at initial exposure, and that the excess mortality is independent of time since last exposure. Wicks et al<sup>186</sup> compared the histological types of 42 bronchogenic cancers in As-exposed smelter workers with those in 42 matched controls; 38 percent of the cancers in the As-exposed workers were adenocarcinomas versus 12 percent in the controls. Cuzik et al<sup>38</sup> analyzed cancer statistics in a cohort of 478 patients who ingested Fowler's solution (potassium arsenite). An excess of fatal and non-fatal skin cancer was noted, but mortality from internal malignancies was not increased. Sunderman<sup>160</sup> cited six published cases of hepatic angiosarcomas in persons with chronic oral exposures to arsenic compounds; in view of the rarity of this tumor, he speculated that arsenic might play an etiologic role. This suggestion was substantiated by reports of eight additional cases of hepatic angiosarcomas in subjects who ingested inorganic arsenic medications or arsenic polluted water.<sup>51,52,144,193</sup>

**Chromium**

As shown in table IV, recent epidemiological studies<sup>6,64,153</sup> confirmed earlier observations that workers who are exposed to chromate compounds have increased risk of lung cancer mortality. Abe et al<sup>4</sup> found that the latent period between initial exposure to chromate dust and detection of lung cancer was significantly shorter in five cases of small cell carcinoma than in 13 cases with squamous cell carcinoma. Based upon a novel method of "probability window analysis," Hill and Ferguson<sup>69</sup> deduced a downward trend in lung cancer risk of workers in a chrome manufacturing plant, paralleling improvements of the working environment. The validity of this statistical approach has been challenged by Braver and Infante.<sup>16</sup> Sarto et al<sup>152</sup> detected statistically significant increases of chromosomal aberrations and sister chromatid exchanges in cultured lymphocytes from CrO<sub>3</sub>-exposed workers in four electroplating factories; the clastogenic effects of
CrO₃ exposure were positively correlated with chromium concentrations in urine specimens from these workers. Ohsaki described an early detection program for lung cancer in chromate workers, involving semiannual sputum cytology and chest X-ray examinations and annual fiberoptic bronchoscopy; the program identified four cases of bronchogenic carcinoma in a small group of exposed men.

**Nickel.**

The literature during 1979 to 1983 includes epidemiological studies of respiratory cancer in three groups of nickel refinery workers, and in four other groups of workers who had various industrial exposures to nickel compounds (table V). Consistent with previous findings, the nickel refinery workers had increased mortality from lung or sinonasal cancers, while the other nickel-exposed workers did not have increased risks of respiratory cancer. Burges found four cases of gastric cancer in a small cohort of Ni-plating workers, versus 0.6 expected (O/E = 6.23, P < 0.005). Burges stressed that his findings should be interpreted cautiously; he urged further cancer mortality studies of workers in the nickel electroplating trade. According to Chovil et al., the histological classification of 43 bronchogenic carcinomas in nickel-refinery workers included 39 squamous cell carcinomas (91 percent), two oat-cell carcinomas (5 percent), and two adenocarcinomas (5 percent). Waksvik and Boysen reported cytogenetic analyses on blood lymphocytes of nickel refinery workers; they observed increased incidence of chromosomal gaps, but no increases in chromosomal breaks or sister-chromatid exchanges.

Boysen and Reith described a gradual multistep progression of dysplastic, metaplastic, and neoplastic lesions in nasal epithelium of nickel refinery workers, based upon light microscopy, transmission electron microscopy, and scanning electron microscopy of
TABLE V

RESPIRATORY CANCER MORTALITY IN NICKEL-EXPOSED WORKERS

<table>
<thead>
<tr>
<th>Authors &amp; Country</th>
<th>Worker Population</th>
<th>Year of First Employment</th>
<th>Lung or Nasal Cancer Site</th>
<th>Obs(0)</th>
<th>Exp(E)</th>
<th>Deaths</th>
<th>O/E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chovil et al. (21) Canada</td>
<td>Ni-refinery (sintering)</td>
<td>1948-62</td>
<td>L</td>
<td>37a</td>
<td>5a</td>
<td>4.26</td>
<td>8.69</td>
</tr>
<tr>
<td>Magnus et al. (108) Norway</td>
<td>Ni-refinery</td>
<td>1916-65</td>
<td>L</td>
<td>82</td>
<td>N</td>
<td>21</td>
<td>0.8</td>
</tr>
<tr>
<td>Enterline &amp; Marsh (46) USA</td>
<td>Ni-refinery</td>
<td>1922-47</td>
<td>L</td>
<td>8</td>
<td>N</td>
<td>2</td>
<td>0.08</td>
</tr>
<tr>
<td>Godbold &amp; Tompkins (60) USA</td>
<td>Ni-diffusion barrier plant</td>
<td>1948-53</td>
<td>L</td>
<td>3</td>
<td>N</td>
<td>0</td>
<td>6.68</td>
</tr>
<tr>
<td>Cuckle et al. (37) England</td>
<td>Soluble Ni production</td>
<td>1933-60</td>
<td>L</td>
<td>13</td>
<td>N</td>
<td>0</td>
<td>9.92</td>
</tr>
<tr>
<td>Cox et al. (35) England</td>
<td>Ni-alloy production</td>
<td>1953-78</td>
<td>L</td>
<td>15</td>
<td>N</td>
<td>0</td>
<td>18.21</td>
</tr>
<tr>
<td>Burges (19) England</td>
<td>Ni-platersb</td>
<td>1945-75</td>
<td>L</td>
<td>1</td>
<td>N</td>
<td>0</td>
<td>1.7</td>
</tr>
</tbody>
</table>

a Includes two workers with primary cancers of both lung and nose.
b P < 0.01

Kotlar et al87 employed a leukocyte adherence inhibition test to assay serum from nickel refinery workers for immune responses to carcinoma antigens. The frequency of positive response against a lung carcinoma antigen was 41 percent in the nickel-exposed workers, compared to 18 percent in controls. In nickel workers with nasal dysplasia, 56 percent gave a positive reaction against the lung carcinoma antigen, compared to 25 percent in nickel workers without dysplasia. Similar trends were found for immune responses to a nasal carcinoma antigen. Kotlar et al87 concluded that the leukocyte adherence inhibition assay may help to identify nickel workers with increased risk of respiratory cancer.

Cadmium

Kjellstrom et al86 analyzed cancer mortality in two groups of cadmium-exposed workers. Among 269 Cd-Ni battery workers, there were two deaths from nasopharyngeal cancer, versus 0.20 expected (O/E = 10.0, P < 0.005) and two deaths from prostatic cancer, versus 1.2 expected, (O/E = 1.67, not significant). Among 94 Cd-Cu alloy workers, there were four deaths from prostatic cancer versus 2.7 expected (O/E = 1.49, not significant). The authors noted that the nasopharyngeal cancers might conceivably be related to nickel exposure and that the prostatic cancers could possibly be connected with cadmium exposure. Armstrong and Kazantis9 studied mor-
tality of cadmium-exposed workers in Britain. In the entire cohort, which included 6995 men exposed to Cd for more than one year between 1942 and 1970, there were 23 deaths from prostatic cancer, versus 23.3 expected (O/E = 0.99, not significant) and 199 deaths from lung cancer, versus 185.6 expected (O/E = 1.07, not significant).

Sorahan and Waterhouse\textsuperscript{159} analyzed mortality in a cohort of 3025 British Ni-Cd battery workers during the period 1946 to 1981. There were eight deaths from prostatic cancer, versus 6.6 expected (O/E = 1.21, \( P < 0.05 \)) and 89 deaths from cancer of the respiratory tract, versus 70.2 expected (O/E = 1.27, \( P < 0.05 \)). Inskip et al\textsuperscript{76} compared the mortality of residents in Shiphams, a village in Somerset, England, with high cadmium levels in soil, versus the mortality of residents in a nearby control village. The study did not reveal excess deaths from lung or prostate cancer in the Shiphams population, although small excesses of hypertensive, cardiovascular, and genitourinary diseases were noted in Shiphams.\textsuperscript{76} Based upon these various studies, the epidemiological evidence that cancer risks may be increased in Cd-exposed persons seems meagre and inconclusive.

**Carcinogenesis Bioassays**

**Arsenic**

Arsenic has been the only metal for which epidemiological evidence of human carcinogenicity was not confirmed by carcinogenesis bioassays in experimental animals.\textsuperscript{56,75,96,136} Four recent studies, summarized in table VI, suggest that carcinogenicity of arsenic compounds in animals may eventually be documented. Ivankovic et al\textsuperscript{78} reported that a single intratracheal instillation of a pesticide mixture that contained Ca\textsubscript{3}(AsO\textsubscript{4})\textsubscript{2}, CuSO\textsubscript{4}, and Ca(OH)\textsubscript{2} produced lung carcinomas in 9 of 15 rats. No lungs tumors developed in the control group that received an intratracheal instillation of saline. Since the investigators did not test the individual ingredients of the pesticide mixture, the evidence for carcinogenicity of Ca\textsubscript{3}(AsO\textsubscript{4})\textsubscript{2} is inferen-

**TABLE VI**

<table>
<thead>
<tr>
<th>Authors</th>
<th>Substances</th>
<th>Animals</th>
<th>Route</th>
<th>Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ivankovic et al.</td>
<td>&quot;Bordeaux</td>
<td>BD-TX rats</td>
<td>itr</td>
<td>Lung carcinomas in 9 of 15 rats</td>
</tr>
<tr>
<td>(78)</td>
<td>mixture&quot;\textsuperscript{a}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rudnai &amp;</td>
<td>As\textsubscript{3}O\textsubscript{3}</td>
<td>CFLP-</td>
<td>sc\textsuperscript{b}</td>
<td>Lung tumors in 63% (vs 18% in controls)</td>
</tr>
<tr>
<td>Borzsonyi (151)</td>
<td></td>
<td>mice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chung &amp; Liu (22)</td>
<td>As-ore</td>
<td>rats</td>
<td>itr</td>
<td>Lung carcinomas in 6 of 41 rats</td>
</tr>
<tr>
<td></td>
<td>dusts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ishinishi &amp;</td>
<td>As\textsubscript{3}O\textsubscript{3}</td>
<td>Syrian</td>
<td>itr</td>
<td>Lung tumors in 5 of 30 hamsters</td>
</tr>
<tr>
<td>Yamamoto (77)</td>
<td></td>
<td>hamsters</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} A mixture of CuSO\textsubscript{4}, Ca(OH)\textsubscript{2}, and Ca\textsubscript{3}(AsO\textsubscript{4})\textsubscript{2}.

\textsuperscript{b} Administration to dams on day 15-18 of gestation and to pups on days 1-3 after birth.
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tial and inconclusive. Rudnai and Borzsonyi\textsuperscript{151} exposed mice to AsO\textsubscript{3} on days 15 to 18 of intrauterine life and on days 1 to 3 post-partum. The lung tumor incidence one year later was 63 percent in the As-treated rats versus 18 percent in controls.

Chung and Liu\textsuperscript{22} reported positive carcinogenesis tests in rats that were observed in three years following intratracheal instillations of As-containing ore dusts; lung cancers were found in three of 20 rats treated with ore that contained 2 percent As, 54 percent Fe, and 0.1 percent Pb, and in three of 21 rats treated with ore that contained 16 percent As, 26 percent Fe, and 16 percent Pb. No lung tumors were detected in 18 controls. The authors did not exclude Fe or Pb as factors that might contribute to the pulmonary neoplasia; they speculated about possible risks of lung cancer in underground tin miners in Yunnan, China, who are exposed to inhalation of the ore dusts.\textsuperscript{22} Ishinishi and Yamamoto\textsuperscript{77} administered AsO\textsubscript{3} to female Syrian hamsters as 15 intratracheal instillation, once weekly for four months. During observations for the life span, pulmonary adenomas occurred in 5/30 hamsters in the treated groups versus 0/35 in the control groups. The authors concluded that AsO\textsubscript{3} is weakly tumorogenic for hamster lung.

**Nickel**

Recent carcinogenesis tests of nickel compounds in rodents are listed in table VII. Sunderman et al.\textsuperscript{168} demonstrated a dose-effect relationship for induction of

<table>
<thead>
<tr>
<th>Authors</th>
<th>Agent</th>
<th>Animals</th>
<th>Route</th>
<th>Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunderman et al. (168)</td>
<td>Ni\textsubscript{3}S\textsubscript{2}</td>
<td>Fischer rats</td>
<td>intra-renal</td>
<td>Renal cancers in 18 of 24 rats at 10 mg dosage</td>
</tr>
<tr>
<td>Oskarsson et al. (132)</td>
<td>Ni\textsubscript{3}S\textsubscript{2}</td>
<td>NMRI mice</td>
<td>im, sc</td>
<td>Local sarcomas in 19 of 32 mice at 10 mg dosage</td>
</tr>
<tr>
<td>Sunderman et al. (171)</td>
<td>Ni\textsubscript{3}S\textsubscript{2}</td>
<td>Pregnant rats</td>
<td>im, on day 6</td>
<td>Local sarcomas in all dams; no excess tumors in progeny</td>
</tr>
<tr>
<td>Hildebrand &amp; Tetaert (68)</td>
<td>Ni\textsubscript{3}S\textsubscript{2}</td>
<td>Rabbits</td>
<td>im</td>
<td>Leiomyosarcomas with myosin light-chains typical of fetal smooth muscle</td>
</tr>
<tr>
<td>Albert et al. (5)</td>
<td>Ni\textsubscript{3}S\textsubscript{2}</td>
<td>Fischer rats</td>
<td>intra-ocular</td>
<td>Retinoblastomas, gliomas, and melanomas</td>
</tr>
<tr>
<td>Yamashiro et al. (189)</td>
<td>Ni\textsubscript{3}S\textsubscript{2}</td>
<td>Fischer &amp; hooded rats</td>
<td>im</td>
<td>Rhabdomyosarcomas, leiomyosarcomas, fibrosarcomas, and lymphosarcomas</td>
</tr>
<tr>
<td>Sunderman &amp; McCully (170)</td>
<td>Ni\textsubscript{3}S\textsubscript{2}</td>
<td>Fischer rats</td>
<td>im</td>
<td>Ni\textsubscript{3}S\textsubscript{2}-carcinogenesis inhibited by simultaneous injection of Mn dust</td>
</tr>
<tr>
<td>Kasprzak et al. (83)</td>
<td>Ni\textsubscript{3}S\textsubscript{2}, Ni(OH)\textsubscript{2}, rats</td>
<td>Wistar rats</td>
<td>im</td>
<td>Sarcomas induced by Ni\textsubscript{3}S\textsubscript{2} and cryst. Ni(OH)\textsubscript{2}, but not by NiSO\textsubscript{4} or amorph. Ni(OH)\textsubscript{2}</td>
</tr>
</tbody>
</table>
renal cancers in rats by intrarenal (ir) injection of nickel subsulfide (Ni$_3$S$_2$) and showed that renal carcinogenesis was antagonized by simultaneous ir administration of Mn dust. Oskarsson et al.\textsuperscript{132} induced sarcomas in mice by parenteral injections of radiolabelled Ni$_3$S$_2$. X-ray diffractometry of Ni-crystals recovered from the injection site revealed gradual conversion of $\alpha$Ni$_3$S$_2$ to $\alpha$Ni$_7$S$_6$ and $\beta$NiS; whole body autoradiography showed progressive mobilization of solubilized $^{63}$Ni and $^{35}$S from the injection site. Sunderman et al.\textsuperscript{171} reported the negative outcome of a transplacental carcinogenesis bioassay of Ni$_3$S$_2$ in Fischer rats. Hildebrand and Tetaert\textsuperscript{68} analyzed myosin light-chains in leiomyosarcomas induced in rabbits by im injection of Ni$_3$S$_2$. The tumoral myosin light-chains were characteristic of fetal smooth muscle cells rather than smooth muscle cells of blood vessels, suggesting that the tumors arose from retrodifferentiated muscle cells rather than from preexisting mesenchymal cells. Albert et al.\textsuperscript{5} observed malignant ocular tumors in 14 of 15 Fischer rats within eight months after an intraocular injection of Ni$_3$S$_2$. The ocular tumors included melanotic melanomas with typical melanosomes, even though the test animals were albino rats with amelanotic uveal melanocytes.

Yamashiro et al.\textsuperscript{189} described the ultrastructural features of 24 tumors induced in rats by im injection of Ni$_3$S$_2$, including rhabdomyosarcomas, leiomyosarcomas, fibrosarcomas, lymphosarcomas, and poorly differentiated sarcomas. Sunderman and McCully\textsuperscript{170} found that Ni$_3$S$_2$ induction of sarcomas in rats was inhibited by combined administration of Mn dust at the same injection site, but was not affected when Ni$_3$S$_2$ was injected in one thigh and Mn dust was injected in the other thigh, indicating that inhibition of Ni$_3$S$_2$-carcinogenesis by Mn dust is a local rather than a systemic effect. Kasprzak et al.\textsuperscript{83} reported that im administration of Ni$_3$S$_2$ or crystalline Ni(OH)$_2$ induced local sarcomas in Wistar rats, whereas colloidal Ni(OH)$_2$ and soluble NiSO$_4$ were not carcinogenic under comparable bioassay conditions; the tumor incidences appeared to be inversely related to the in vitro dissolution rates of the nickel compounds.

In addition to the studies listed in table VII, an extensive series of investigations has been conducted by Sunderman and associates\textsuperscript{89,90,165,167,169,172} in an attempt to relate physical, chemical, and biological properties of nickel compounds to their carcinogenic activities. Marked differences were observed in the incidences of sarcomas in male Fischer rats within two years after im injection of 18 nickel compounds at equivalent dosages (14 mg Ni per rat).\textsuperscript{165} The nickel compounds fell into five categories: compounds in Class A (Ni$_4$FeS$_4$, $\alpha$Ni$_3$S$_2$, $\beta$NiS) induced sarcomas at the injection site in 100 percent of the rats; compounds in Class B (NiO, Ni$_3$Se$_2$, NiAsS, NiS$_2$, Ni$_5$As$_2$) induced sarcomas in 85 to 93 percent of the rats; compounds in Class C (Ni dust, NiSb, NiTe, NiSe, and Ni$_{11}$As$_8$) induced sarcomas in 50 to 65 percent of the rats; compounds in class D (amorphous NiS, NiCrO$_4$) induced local sarcomas in 6 to 12 percent of the rats; and compounds in Class E (NiAs, NiTiO$_3$, and NiFe alloy) did not induce any sarcomas.\textsuperscript{165,169,172} No sarcomas occurred at the injection site in 84 control rats that received im injections of the vehicles.

Sarcoma incidences in rats that received im injections of the 18 nickel compounds were significantly correlated ($P = 0.02$) with the mass-fractions of nickel in the respective compounds.\textsuperscript{165} Sarcoma incidences were not significantly related to dissolution rates of the nickel compounds in rat serum or renal cytosol,\textsuperscript{90} or to the susceptibilities of the compounds...
to phagocytosis by rat peritoneal macrophages in vitro. Striking rank correlation (P < 0.0001) was evident between the sarcoma incidences and the capacities of the Ni-compounds to induce erythrocytosis after intrarenal (ir) administration to rats, indicating that derepression of the gene that regulates renal production of erythropoietin can serve as an index of carcinogenic activity of nickel compounds in rats, and suggesting that Ni-stimulation of erythropoietin production and oncogenesis may, in some way, be related. Consistent with this speculation, ir injection of Ni₃S₂ causes prompt induction of erythropoietin-mediated erythrocytosis and delayed induction of renal cancers; both phenomena are inhibited by administration of Mn dust.

**Platinum**

In view of the electrophilic reactivities of cis-Pt[II] coordination complexes towards cellular nucleophiles, and the strongly mutagenic effects of cis-Pt[II] compounds in vitro, Leopold et al tested the carcinogenic activities of several cis-platinum compounds in rodents. Repeated ip doses of cis-dichlorodiammine-platinum, with concurrent and subsequent topical applications of croton oil, induced skin papillomas in 50 percent of female CD-1 mice, while mice treated only with the platinum complex or only with croton oil developed no papillomas. Sarcomas developed in 35 percent and 25 percent, respectively, of male Fischer rats that received multiple sc injections of cis-dichlorobis(cyclopentylamine)-platinum[II] and cis-dichlorobis(pyrrrolidine) - platinum[II]. Based upon these findings Leopold et al cautioned that treatment of patients with platinum antitumor complexes may impose a risk of induction of second tumors in long-term survivors.

**Cadmium**

Loser (105) reported a negative carcinogenesis bioassy of CdCl₂ in Wistar rats. Groups of male and female rats were fed diets containing 1, 3, 10, or 50 ppm of Cd[II], respectively, for two years. Cadmium treatment did not increase the total number of tumors or induce any specific types of neoplasms, although the highest level tested resulted in non-neoplastic adverse effects. This study is important, since it remedies the inadequacies of earlier oral carcinogenesis tests of Cd compounds in rats. Oliges et al exposed Wistar rats to continuous inhalation of CdCl₂ for 18 months, with observation for 13 months thereafter. Primary lung carcinomas were found in 70 percent of rats at the 50 μg per m³ exposure level; 53 percent at 25 μg per m³; and 15 percent at 12.5 μg per m³. No primary lung tumors were found in control rats. These studies reveal a dose-dependent incidence of lung carcinomas after inhalation of CdCl₂ in rats.

**Tin**

The National Cancer Institute Bioassay Program recently completed a carcinogenesis bioassy of Sn[II] in rodents, conducted by feeding diets containing 1,000 or 2,000 ppm of SnCl₂ to groups of Fischer rats and B6C3F1/N mice of each sex for two years. SnCl₂ was judged not to be carcinogenic for either rats or mice, although the incidence of C-cell tumors of the thyroid gland seemed to be increased in male rats.

**Bacterial Mutagenesis**

**Arsenic**

Contrary to a previous report by Nishioka, recent studies by Rossman et
al47–149 and Simon158 indicate that As[III] does not induce mutations in tryptophan auxotrophic strains of E. coli (table VIII). Rossman149 observed, however, that As[III] enhances the susceptibility of E. coli (WP2 strain) to mutagenic effects of ultra-violet radiation. This co-mutagenic effect of As[III] occurs only in the excision-proficient strain, WP2, and is absent in strains deficient in excision-repair, indicating that As[III], like caffeine, interferes with excision repair.149 Consistent with earlier findings of Lofroth and Ames,104 Tiedemann and Einbrodt174 reported negative results for As[III] and As[V] in Ames’s Salmonella microsome test. Nishioka120 and Kanematsu et al81 observed positive results for As[III] in the “rec-assay” in B. subtilis, but Simon158 obtained negative results using the same tester strains and procedures. These conflicting data are not included in table VIII, since the rec-assay is not, strictly speaking, a test for mutagenesis. The rec assay is actually a test for DNA damage, based upon the observation that a substance is more toxic for recombination-repair deficient (rec−) than for wild (rec+) strains of B. subtilis.

**Chromium**

Flessel53 concluded that Cr[VI] is the most mutagenic metal ion in Ames’s Salmonella microsome assay, while Cr[III] has little, if any, mutagenic activity in this system. The genotoxicity of Cr[VI] in S. typhimurium involves frame-shift and base-repair mutations. DeFlora et al39 and Venier et al180 tested 28 industrial chromium compounds for mutagenicity in S. typhimurium; the mutagenic potencies of the Cr[VI] compounds were of comparable magnitude, excepting two water-insoluble compounds, chromium hexacarbonyl and chrome yellow; on the other hand, Cr[III] compounds were all inactive, unless they were contaminated with Cr[VI]. These results, indicating selective mutagenicity of Cr[VI] and Cr[III] compounds in S. typhimurium, are in agreement with those obtained with trp− strains of E. coli.180 Reduction of Cr[VI] to Cr[III] by incubation with rat liver microsomes, using either NADPH or NADH as cofactors, almost completely eliminates mutagenic activity.180 Witmer et al187 showed that the sensitivity for detecting mutagenicity of

<table>
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<tr>
<th>Authors</th>
<th>Bacteria</th>
<th>Results</th>
</tr>
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<tbody>
<tr>
<td>Rossman et al. (147-149)</td>
<td>E. coli</td>
<td>As[III] blocks excision repair (co-mutagenic).</td>
</tr>
<tr>
<td>Simon (158)</td>
<td>E. coli</td>
<td>As[III] neg.</td>
</tr>
<tr>
<td>Tiedemann &amp; Einbrodt (174)</td>
<td>S. typhimurium</td>
<td>As[III] and As[V] neg.</td>
</tr>
<tr>
<td>Flessel (53)</td>
<td>E. coli</td>
<td>Cr[VI] pos., Cr[III] neg.</td>
</tr>
<tr>
<td>DeFlora et al. (39)</td>
<td>S. typhimurium</td>
<td>Cr[VI] pos., Cr[III] neg.</td>
</tr>
<tr>
<td>Pikalek &amp; Necasek (137)</td>
<td>Corynebacterium</td>
<td>Ni[II] pos. in homoserine revertant assay</td>
</tr>
</tbody>
</table>
Cr\[VI\] in Ames’s Salmonella microsome test can be enhanced by lowering the salt concentrations, varying the histidine concentration, and prolonging the incubation period.

Nickel

As reviewed by Sunderman,\textsuperscript{164} nickel compounds are not mutagenic in the \textit{S. typhimurium} or \textit{E. coli} test systems. Pikalek and Necasek\textsuperscript{137} recently showed that Ni\[II\] is mutagenic for a homoserine-dependent strain of Corynebacterium (sp. 887 hom), using a simplified fluctuation test as well as the clone method. Their identification of a suitable bacterial strain for mutagenesis tests of nickel compounds will facilitate research on molecular mechanisms of nickel genotoxicity.

Other Metals

Kanematsu et al\textsuperscript{81} performed rec-assays in \textit{B. subtilis} on 127 metal compounds, in order to test their DNA-damaging capacities. Certain compounds of As, Be, Cd, Co, Cr, Cs, Hg, Ir, Mo, Os, Pt, Rh, Sb, Se, Te, Tl, and V were positive, whereas the tested compounds of Ag, Al, Au, Ba, Bi, Ca, Cu, Fe, Ga, In, K, La, Mg, Mn, Nb, Ni, Pb, Rb, Ru, Si, Sn, Sr, Ti, Ta, and Y were negative. Reverse mutation assays with \textit{E. coli} and \textit{S. typhimurium} strains demonstrated that certain compounds of Rh, Te, and Pt are potent mutagens in bacteria.\textsuperscript{78}

Mammalian Cell Mutagenesis

Miyaki et al\textsuperscript{113} examined the mutagenicity of Be\[II\], Co\[II\], Mn\[II\], and Ni\[II\] in cultured V79 Chinese hamster cells at the hypoxanthine-guanine phosphoribosyl transferase (HGPRTase) locus, by selecting for resistance to 8-azaguanine (table IX). The selection for mutation is based on the presence of HGPTRase activity in wild-type cells, enabling them to convert 8-azaguanine, a purine analog, to toxic metabolites that cause cell death; mutants escape the lethal effect of 8-azaguanine, since loss of HGPRTase activity renders them incapable of catalyzing the detrimental metabolism. Treatment with beryllium caused six-fold increase in resistance to 8-azaguanine; cobalt, manganese, and nickel increased the resistance two to four-fold.\textsuperscript{113} Miyaki et al\textsuperscript{113} noted that cobalt and nickel were so toxic that it was difficult to detect the induced mutations. They speculated that binding of metal cations to nucleotide bases renders DNA susceptible to strand-scission or to elimination of bases by an unknown nuclease, resulting in mutations during the repair process. Hsie et al\textsuperscript{72} used a similar technique to test the mutagenicity of 14 metal compounds in cultured Chinese hamster ovary cells, using 6-thioguanine as the purine analog. Preliminary results showed that cis-Pt(NH\textsubscript{3})\textsubscript{2}Cl\textsubscript{2}, Ag\[I\], Be\[II\], Cd\[II\], Cu\[II\], Fe\[II\], Mn\[II\], Ni\[II\], Pb\[II\], and Zn\[II\] are mutagenic, whereas Mg\[II\], Rb\[I\], Se\[IV\], and Ti\[IV\] are non-mutagenic. Hsie et al\textsuperscript{72} cautioned that these results need confirmation, since results of mutagenesis assays are influenced by variations in ionic composition of the medium and the physiological state of the cells during treatment.

Amacher and Paillet\textsuperscript{7} tested eight metal compounds for induction of trifluorothymidine resistant mutants at the thymidine kinase locus in mouse lymphoma cells. In cells exposed to varied concentrations of each compound for 3 h, Cd\[II\], Ni\[II\], and trans-Pt(NH\textsubscript{3})\textsubscript{2}Cl\textsubscript{2} consistently produced dose-dependent increases in the absolute number of mutants; As[V], Co\[II\], Mg\[II\], and Zn\[II\] gave negative responses. Oberly et al\textsuperscript{126} tested eleven metal compounds for mutagenic effects at the thymidine kinase locus in mouse lymphoma cells, in-
TABLE IX

MUTAGENESIS ASSAYS OF METAL CATIONS IN MAMMALIAN CELLS

<table>
<thead>
<tr>
<th>Authors</th>
<th>System</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miyaki et al.</td>
<td>HGPRT assay(^a) in</td>
<td>(\text{Be}[\text{II}], \text{Co}[\text{II}], \text{Mn}[\text{II}], \text{Ni}[\text{II}]) pos.</td>
</tr>
<tr>
<td>(113)</td>
<td>Chinese hamster V79 cells</td>
<td></td>
</tr>
<tr>
<td>Hsie et al.</td>
<td>HGPRT assay(^a) in</td>
<td>(\text{Ag}[\text{I}], \text{Be}[\text{II}], \text{Cd}[\text{II}], \text{Cu}[\text{II}], \text{Fe}[\text{II}])</td>
</tr>
<tr>
<td>(72)</td>
<td>CHO-K1-BH4 cells</td>
<td>(\text{Mn}[\text{II}], \text{Ni}[\text{II}], \text{Pb}[\text{II}], \text{Zn}[\text{II}], \text{cis}-\text{Pt}[\text{II}]) pos; (\text{Mg}[\text{II}], \text{Rb}[\text{I}], \text{Se}[\text{IV}], \text{Ti}[\text{IV}]) neg.</td>
</tr>
<tr>
<td>Amacher &amp; Paillet</td>
<td>TK assay(^b) in</td>
<td>(\text{Cd}[\text{II}], \text{Ni}[\text{II}], \text{trans-Pt}[\text{II}]) pos;</td>
</tr>
<tr>
<td>(7)</td>
<td>L 51784 mouse lymphoma cells</td>
<td>(\text{As}[\text{V}], \text{Co}[\text{II}], \text{Mg}[\text{II}], \text{Zn}[\text{II}]) neg.</td>
</tr>
<tr>
<td>Oberly et al.</td>
<td>TK assay(^b) in</td>
<td>(\text{As}[\text{III}], \text{As}[\text{V}], \text{Cd}[\text{II}], \text{Cr}[\text{VI}], \text{Hg}[\text{II}])</td>
</tr>
<tr>
<td>(126)</td>
<td>L 51784 mouse lymphoma cells</td>
<td>(\text{Mn}[\text{II}], \text{Pb}[\text{II}]) pos; (\text{Al}[\text{III}], \text{Mg}[\text{II}], \text{Na}[\text{I}]) neg.</td>
</tr>
</tbody>
</table>

\(^a\) Hypoxanthine-guanine phosphoribosyl transferase assay; mutations resistant to 8-azaguanine or 6-thioguanine.

\(^b\) Thymidine kinase assay; mutations resistant to trifluorothymidine.

including, in certain experiments, activation with rat liver microsomes and NADP as a cofactor. Strongly positive responses were obtained with \(\text{Cd}[\text{II}], \text{Cr}[\text{VI}], \text{and Mn}[\text{II}]\); weakly positive responses (i.e., two- to three-fold increases in mutation frequency) were obtained with \(\text{Hg}[\text{II}], \text{As}[\text{III}], \text{As}[\text{V}], \text{and Pb}[\text{II}]\); negative responses were obtained with \(\text{Al}[\text{III}], \text{Mg}[\text{II}], \text{and Na}[\text{I}]\).

Chromosomal Damage

Overview

The literature prior to 1979 on clastogenic effects of metals has been summarized by Sunderman, Kazantis and Lilly, and Flessel. Four groups of investigators have recently performed extensive investigations of chromosomal damage and sister chromatid exchanges induced by in vitro exposures of mammalian cells to metal compounds. Umeda and Nishimura exposed FM3A cells from a C3H mouse mammary carcinoma to \(\text{Cr}[\text{III}], \text{Cr}[\text{VI}], \text{Ni}[\text{II}], \text{Mn}[\text{II}], \text{Cd}[\text{II}], \text{and Hg}[\text{II}]\) compounds at various concentrations for 24 or 48 hrs. \(\text{Cr}[\text{VI}]\) compounds induced numerous chromosomal breaks and exchanges, while \(\text{Cr}[\text{III}]\) was inactive; \(\text{NiS}\) caused a definite increase of aberrations, while the effects of \(\text{NiCl}_2\) and \(\text{Ni}(\text{C}_2\text{H}_3\text{O}_2)_2\) were equivocal; \(\text{Mn}[\text{II}]\) and \(\text{Mn}[\text{V}]\) compounds induced a few aberrations; \(\text{Cd}[\text{II}]\) and \(\text{Hg}[\text{II}]\) compounds were not clastogenic.

Larramendy et al. exposed Syrian hamster embryo cells and human lymphocyte cultures to various concentrations of five metal compounds for 24 hrs prior to examination for chromosomal damage and sister chromatid exchanges. \(\text{As}[\text{III}], \text{As}[\text{V}], \text{Be}[\text{II}], \text{and Ni}[\text{II}]\) caused chromosomal damage and increased the frequencies of sister chromatid ex-
changes in human and hamster cells; W[VI] was negative in both test systems (table X). Larramendy et al reasoned that, although the formation of chromosomal aberrations and sister chromatid exchanges involves different mechanisms, carcinogen interaction with DNA is probably responsible for both; since sister chromatid exchanges are compatible with cell survival, they seem more relevant to carcinogenesis, whereas chromosomal aberrations are primarily associated with cell death. Ohno et al exposed Don Chinese hamster cells to various metal compounds for 28 hrs prior to examination for sister chromatid exchanges; under these conditions, As[III], As[V], Ni[II], and Cr[VI] compounds caused significantly increased numbers of sister chromatid exchanges, while Cr[III], Ti[III], Fe[II], Fe[III], Cd[II], Sn[II], and Hg[II] did not. Anderson investigated the effects of metals on sister chromatid exchanges in human lymphocytes and in a macrophage cell line, P388D1, which originated from a mouse lymphoma; in vitro exposures to the test compounds ranged from 24 to 72 hrs. Increased incidences of sister chromatid exchanges were observed in human lymphocytes exposed to As[III], Cd[II], Co[II], Cr[VI], Hg[II], Mn[II], Ni[II], and Pb[II]. Similar results were observed in P388D1 cells, except that Cr[III] was strongly positive and Be[II] was weakly positive. Since human lymphocytes exclude Cr[III], whereas P388D1 cells take up Cr[III] by phagocytosis, these observations are consistent with the concept, discussed subsequently, that Cr[III] may be the ultimate carcinogenic form of chromium.

**Arsenic**

Zanzoni and Jung, Wen et al, and Crossen confirmed that in vitro exposures to As[III] or As[V] compounds induce sister chromatid exchanges in human lymphocytes. Based upon tests with lymphocytes from 16 donors, Crossen concluded that individuals vary considerably in their sister chromatid responses to arsenic. Wen et al noted significantly higher frequency of baseline sister chromatid exchanges in cultured lymphocytes from patients with blackfoot disease, compared to healthy controls; lymphocytes from the patients and controls were equally susceptible to induction of sister chromatid exchanges following in vitro exposure to As[III].

**Chromium**

Levis, Majone, and coworkers studied the clastogenic effects of numerous Cr[VI] and Cr[III] compounds on cell cultures derived from Chinese hamster ovary (CHO), baby hamster kidney (BHK), or mouse spleen T-lymphocytes. Water-soluble compounds of Cr[VI] and Cr[III] induced mitotic delays and chromosomal aberrations; Cr[III] was incapable of inducing sister chromatid exchanges, only Cr[VI] being active. Levis and Majone concluded that the state of oxidation is the most important parameter that affects the mutagenic activity of chromium compounds; other properties, such as solubility in water, ability to penetrate cell membranes, and intracellular stability of Cr[VI] ion may account for differences between the results of long-term carcinogenesis tests and short-term mutagenesis tests. Further evidence to this point was obtained by Elias et al, who studied the induction of sister chromatid exchanges in Chinese hamster V79 cells exposed to soluble CrCl3 and insoluble Cr2O3. Both of these Cr[III] compounds induced dose-dependent increases in sister chromatid exchanges, up to two-fold (CrCl3) and four-fold (Cr2O3) over control levels. Elias et al attributed the pronounced effect of Cr2O3 to cellular uptake of particles by phagocytosis; the
### TABLE X

**INDUCTION OF SISTER CHROMATID EXCHANGES (SCE)**

**BY IN VITRO EXPOSURES TO METALS**

<table>
<thead>
<tr>
<th>Authors</th>
<th>Cells</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larramendy et al. 93</td>
<td>SHE cells &amp; human lymphocytes</td>
<td>As[III], As[V], Be[II], Ni[II] pos.; W[VI] neg.</td>
</tr>
<tr>
<td>Ohno et al. 127</td>
<td>Don-CHO cells</td>
<td>As[III], As[V], Cr[VI], Ni[II] pos.; Cd[II], Cr[III], and others neg.</td>
</tr>
<tr>
<td>Andersen (8)</td>
<td>Human lymphocytes &amp; P388D₁ cells</td>
<td>As[III], Cd[II], Cr[VI], Co[II], Hg[II], Mn[II], Ni[II], Pb[II] pos. in lymphocytes; similar results in P388D₁ cells, except Cr[III] and Be[II] pos.</td>
</tr>
<tr>
<td>Zanzoni &amp; Jung 194</td>
<td>Human lymphocytes</td>
<td>As[III] gave dose-dependent increase in SCE.</td>
</tr>
<tr>
<td>Wen et al. 185</td>
<td>Human lymphocytes</td>
<td>As[III] increased SCE in lymphocytes from patients with blackfoot disease.</td>
</tr>
<tr>
<td>Crossen (36)</td>
<td>Human lymphocytes</td>
<td>As[III], As[V] pos.</td>
</tr>
<tr>
<td>Levis, Majone, et al. (100,101,109,110)</td>
<td>BHK, CHO, &amp; T-lymphocytes</td>
<td>Cr[VI] pos; Cr[III] neg.</td>
</tr>
<tr>
<td>Elias et al. 45</td>
<td>V79 Chinese hamster cells</td>
<td>Cr[III] pos. (as CrCl₃ or Cr₂O₆)</td>
</tr>
<tr>
<td>Nishimura &amp; Umeda 119</td>
<td>FM3A cells</td>
<td>Ni[II] pos. after reincubation in control medium for 1-2 days</td>
</tr>
<tr>
<td>Wulf 188</td>
<td>Human lymphocytes</td>
<td>Ni[II], Pb[II] pos.</td>
</tr>
<tr>
<td>Saxholm et al. 154</td>
<td>Human lymphocytes</td>
<td>Ni₃S₂ pos.</td>
</tr>
<tr>
<td>Newman et al. 118</td>
<td>Human lymphocytes</td>
<td>Ni[II] pos.</td>
</tr>
<tr>
<td>Popescu et al. 139</td>
<td>SHE cells</td>
<td>cis-Pt[II] pos.</td>
</tr>
</tbody>
</table>
particles presumably dissolve gradually in the cytoplasm and release Cr$^{3+}$, which enters the nucleus and reacts with DNA or related macromolecules.

Nickel

Nishimura and Umeda$^{119}$ compared the effects of four nickel compounds (NiCl$_2$, Ni(CH$_3$COO)$_2$, K$_2$Ni(CN)$_4$, and NiS) on induction of chromosomal aberrations in cultured FM3A cells. The four nickel compounds were similarly incorporated in the cells and elicited similar inhibitory effects on synthesis of protein, RNA, and DNA. The maximum incidence of chromosomal aberrations, including breaks, exchanges, and fragmentation, was observed after reincubation in control medium for one or two days, by which time the cells were entering their second or third division. Wulf$^{188}$ found that in vitro exposure of human lymphocytes to Ni[II] (as NiSO$_4$) at concentrations as low as $2 \times 10^{-6}$ mol per litre, or Pb[II] (as PbSO$_4$) at concentrations as low as $2 \times 10^{-5}$ mol per litre cause significant increases in sister chromatid exchanges. Saxholm et al$^{154}$ noted a small but statistically significant increase of sister chromatid exchanges in human lymphocytes exposed in vitro to crystalline Ni$_3$S$_2$. Newman et al$^{118}$ observed increased sister chromatid exchanges in human lymphocytes exposed to NiCl$_2$ in the range from $10^{-4}$ to $10^{-6}$ mol per litre; the incidence of exchanges appeared to be disproportionately enhanced in chromosomes 1 and 2, and in the B group.

Platinum

Popescu et al$^{139}$ reported dose-dependent induction of sister chromatid exchanges in Syrian hamster fetal cells exposed in vitro to cis-Pt(NH$_3$)$_2$Cl$_2$.

Mammalian Cell Transformation

Mammalian cell transformation assays of metal compounds have been reviewed by Heck and Costa. Casto et al$^{120}$ and DiPaolo and Casto$^{41}$ evaluated 45 metal salts for their capacities to induce morphological transformation of Syrian hamster fetal cells in vitro (table XI). Positive transformation assays were obtained with Ag[II], As[III], Be[II], Cd[II], Co[II], Cr[VI], Cu[II], Fe[II], Hg[II], Mn[II], Ni[II], Pb[II], Pt[II], Pt[IV], Sb[III], Ti[I], and Zn[II]; negative results were obtained with Al[III], Ba[II], Ca[II], Li[I], Mg[II], Na[I], Sr[II], Ti[IV], and W[VI]. The percentage of transformed cells obtained with Ni$_3$S$_2$ was the highest of all the metal compounds; under the same conditions amorphous NiS gave negative results in the transformation assay. When Ni[II], Be[II], Cd[II], or Cr[VI] 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. Costa et al$^{32,33}$ showed that morphological transformation of Syrian hamster embryo cells by Ni$_3$S$_2$ is dose-dependent and can be prevented by addition of Mn dust to the incubation medium; several clones of Ni$_3$S$_2$-transformed cells produced fibrosarcomas following sc implantation in nude mice. Costa and coworkers$^{28,30,31,34}$ studied particle uptake of six crystalline compounds (Ni$_3$S$_2$, NiS, Ni$_3$Se$_2$, CuS, CdS, and CoS$_2$) and four amorphous compounds (NiS, CuS, CdS, and CoS) by Chinese hamster ovary cells. The cells avidly phagocytized the crystalline particles, whereas they engulfed few amorphous particles, suggesting that the greater transforming activities of the crystalline compounds may be attributed to differences in their rates of cellular uptake.$^{29-31,34}$

Abbrachio et al$^2$ presented evidence
that the phagocytosis and transforming activity of crystalline metal sulfide particles are related to their negative surface changes. By means of video time-lapse microscopy, Evans et al\textsuperscript{50} described endocytosis and intracellular translocation of crystalline NiS particles in Chinese hamster ovary cells. After 24 to 48 hrs, the NiS particles become fixed in the perinuclear region, oftentimes situated within cytoplasmic vacuoles. Abbrachio et al\textsuperscript{3} showed that crystalline 63NiS particles are phagocytized by Chinese hamster ovary cells and gradually dissolve in the cytosol; a portion of the liberated 63Ni becomes localized in nuclei. Costa et al\textsuperscript{128,129} found that soluble NiCl$_2$ induces morphological transformation of Syrian hamster embryo cells; its potency averages two-fifths that of crystalline NiS, probably owing to higher cellular uptake of crystalline NiS by phagocytosis, compared to lower uptake of ionic nickel under the usual conditions of incubation.

Abbracchio et al\textsuperscript{1} noted that cells incubated in a minimal medium accumulate 10-fold more 63Ni[II] than when incubated in complete medium that contains histidine, cysteine, and fetal bovine serum. Saxholm et al\textsuperscript{154} demonstrated that Ni$_3$S$_2$ induces morphological transformation of C3H/10T 1/2 cells; scanning electron microscopy revealed an oncogenic marker, long microvilli, on the transformed cells. Hansen and Stern\textsuperscript{61} compared the activity of five nickel compounds (Ni dust, Ni$_3$S$_2$, Ni$_2$O$_3$, NiO, and Ni(C$_2$H$_3$O$_2$)$_2$) for \textit{in vitro} transformation of BHK-21 cells. Although the nickel compounds varied substantially in their transforming potencies, the compounds produced the same number of transformed colonies at the same degree of toxicity (e.g., 50 percent survival). The authors concluded that the sole property that determines the transforming potency of nickel compounds is the intracellular bioavailability of Ni[II].\textsuperscript{61}

Costa and Mollenhauer,\textsuperscript{30,31} Rivedal and Sanner,\textsuperscript{142,143} and Uziel and Butler\textsuperscript{178}
observed synergistic effects of nickel compounds and benz(a)pyrene (BP) on morphological transformation of Syrian hamster embryo cells. Costa and Mollenhauer found that pretreatment of the cells with BP enhances cellular uptake of Ni3S2 particles. According to Rivedal and Sanner, combined treatment of Syrian hamster embryo cells with NiSO4 and BP results in a transformation frequency of 10.7 percent, compared to 0.5 percent and 0.6 percent for the individual substances. No synergistic effect could be detected between NiSO4 and methylcholanthrene. Rivedal and Sanner found that Ni[II], Cd[II], and Cr[VI] exert synergistic effects on BP-transformation of Syrian hamster embryo cells, while Cr[III] and Zn[II] do not. These in vitro observations are consistent with an earlier report by Maenza et al. of carcinogenic synergism between Ni3S2 and BP following administration to Fischer rats.

**Microsomal Metabolism**

Chromium[VI], as chromate or dichromate, is readily taken up by bacteria and eukaryotic cells, while Cr[III] is less capable of traversing cell membranes. Differences in cellular penetration of Cr[VI] and Cr[III] may, in part, account for the observed disparities in mutagenic and carcinogenic activities of chromium compounds. Jennette recently showed that incubation of chromat with rat liver microsomes and NADPH leads to formation of a stable reactive intermediate, Cr[V]; hence Cr[V] rather than Cr[III] may represent the ultimate electrophilic form of chromium that reacts with critical nucleophilic targets to initiate carcinogenesis. Jennette and coworkers demonstrated that microsomal reduction of Cr[VI] is an enzymatic process that requires NADPH or NADH; the process apparently involves cytochrome P-450, NADPH-cytochrome P-450 reductase, and protonated thiol groups (e.g., glutathione, cysteine). These findings suggest that microsomal reduction of Cr[VI] to an electrophilic reactant may be a critical step in the initiation of chromium carcinogenesis. Microsomal metabolism may play a role in the activation of other carcinogenic metals, since Lee et al. observed release of Ni[II] during in vitro incubation of Ni3S2 with rat liver microsomes, calf thymus DNA, and NADPH. Under these experimental conditions, microsomes evidently mediate the binding of Ni[II] to DNA by formation of a ternary protein-Ni-DNA complex.

**Binding to DNA and Nuclear Proteins**

Bryan reviewed the literature on nuclear localization of toxic metals, emphasizing methodological problems from metal contamination or reassociation during nuclear isolation procedures.Sigee and Kearns, using X-ray microanalysis, demonstrated Ni, Cu, Zn, and Fe in dinoflagellate chromatin, associated with high molecular weight proteins and nucleic acids (table XII). Kovacs and Darvas, using dimethylglyoxime staining, showed that Ni is localized in centrioles of HeLa cell cultures. Hui and Sunderman found 0.2 to 2.2 mol 63Ni per mol of DNA nucleotides in DNA isolated from liver and kidney of rats treated with 63NiCl2 or 63Ni(CO)4. Lee et al. measured in vitro binding of Ni to calf thymus DNA incubated in the presence of Ni3S2, rat liver microsomes, and NADPH; the saturation binding value was 0.42 mol Ni per mol of DNA nucleotides. Ciccarelli and Wetterhahn demonstrated Ni[II]-nucleic acid-histone complexes in liver and kidney of NiCO3-treated rats. They proposed that nickel may initiate DNA damage by forming a covalent Ni-DNA complex, which appears to be associated with histone proteins.

Ono et al. analyzed trace metal con-
TABLE XII
BINDING OF METALS TO DNA AND NUCLEAR PROTEINS

<table>
<thead>
<tr>
<th>Authors</th>
<th>System</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sigee &amp; Kearns (157)</td>
<td>Dinoflagellates</td>
<td>Ni, Cu, Zn, and Fe are present in chromatin</td>
</tr>
<tr>
<td>Kovac &amp; Darvas (88)</td>
<td>HeLa cells</td>
<td>Histochemical localization of Ni in centrioles</td>
</tr>
<tr>
<td>Hui &amp; Sunderman (73)</td>
<td>Rats treated with $^{63}$Ni(CO)$_4$ or $^{63}$NiCl$_2$</td>
<td>$^{63}$Ni-bound to DNA in liver and kidney (0.3-2.2 mol Ni/mol DNA nucleotides)</td>
</tr>
<tr>
<td>Lee et al. (94)</td>
<td>In vitro binding of Ni to calf thymus DNA</td>
<td>In presence of liver microsomes and NADPH, DNA binds Ni$^{[II]}$ (0.4 mol Ni/mol DNA nucleotides)</td>
</tr>
<tr>
<td>Ono et al. (131)</td>
<td>Normal &amp; regenerating rat liver</td>
<td>Cr &amp; Ni contents are 11 &amp; 18-fold higher in nucleoli than nuclei</td>
</tr>
<tr>
<td>Parker &amp; Stevens (133)</td>
<td>In vitro binding of Be to rat liver nuclei</td>
<td>Be$^{[II]}$ binds to nucleoli and a non-histone acidic nuclear protein</td>
</tr>
</tbody>
</table>

Centrations in nuclei and nucleoli of normal and regenerating rat liver. The contents of Ca, Cu, Mn and Zn in nuclei were less than 3 percent of those in whole cells, but the contents of Cr and Ni in nuclei were 20 to 30 percent of those in whole cells. The metal contents of nucleoli, expressed per mg of protein, ranged from three-times (Zn) to 11-times (Cr) and 18-times (Ni) those in nuclei. The Cr and Ni that was bound to nucleoli was more resistant than the other metals to treatment with nuclease.

Parker and Stevens$^{133}$ demonstrated that in vitro exposures of rat liver nuclei to Be$^{[II]}$ results in binding of Be$^{[II]}$ to a highly phosphorylated constituent of non-histone proteins. Perry et al.$^{135}$ showed that exposure of rat hepatoma cells in tissue culture to Be$^{[II]}$ ($1 \times 10^{-6}$ mol per litre) reduces glucocorticoid induction of tyrosine transaminase activity by 50 percent, but does not affect induction of the enzyme by insulin or cyclic-AMP. Growth of the hepatoma cells is not affected by Be$^{[II]}$ under the experimental conditions. Perry et al.$^{135}$ speculated that impaired ability of Be$^{[II]}$-treated cells to respond to a specific regulator of gene expression may point to a possible mechanism of beryllium carcinogenesis. Olson$^{130}$ reviewed the effects of metal ions on phosphorylation of nuclear proteins, including his remarkable finding that in vitro exposure to Zn$^{[II]}$ influences phosphorylation of H1-histone in tissue cultures of rat hepatoma cells. After incubation for eight hrs in medium containing ZnCl$_2$ ($1 \times 10^{-3}$ mol per litre), 85 to 90 percent of H1-histone was phosphorylated, as opposed to 65 percent in untreated cells. Furthermore, five of the seven serine residues in H1-histone were phosphorylated, as opposed to only two in untreated cells. Hyperphosphorylation of H1-histone was attended by increased susceptibility of the chromatin to digestion by micrococcal
nuclease and by diminished stability of oligonucleosomal fragments to thermal denaturation and salt disassociation. These observations illustrate a molecular mechanism whereby metal ions can influence chromatin structure.

**DNA Strandbreaks and Crosslinks**

Zwelling et al.\textsuperscript{195,196} demonstrated DNA-protein and DNA-interstrand crosslinks in L1210 mouse leukemia cells and V79 Chinese hamster cells treated with cis- and trans-Pt\textsuperscript{II}-dichlorodiammine (cis-Pt and trans-Pt) (table XIII). Fornace and Little\textsuperscript{54} showed that trans-Pt produces DNA-protein crosslinks in mouse C3H-10T\textsubscript{1/2} and 3T3 cells and induces morphological transformation in both cell types. Fornace et al.\textsuperscript{55} reported that \textit{in vitro} exposure to Cr\textsuperscript{VI} compounds induces DNA-protein crosslinks in several types of mammalian cells, and that exposure to Cr\textsuperscript{III} compound induces DNA-protein crosslinks in isolated nuclei. These data are consistent with the hypothesis that Cr\textsuperscript{VI} traverses the cell membrane into the cytoplasm, where it is reduced to Cr\textsuperscript{III}, and that Cr\textsuperscript{III} enters the nucleus, where it forms stable DNA-protein linkages. Fornace et al.\textsuperscript{55} suggested that the linkage of DNA to protein may influence DNA polymerase activity during DNA replication and repair, with resultant mutagenic and carcinogenic consequences. Tsapakos et al.\textsuperscript{176} identified DNA-protein crosslinks in liver and kidney nuclei from rats treated with Cr\textsuperscript{VI}. Ciccarelli and coworkers\textsuperscript{23,24} demonstrated DNA-protein crosslinks and DNA strandbreaks in kidney nuclei from rats treated with NiCo\textsubscript{3}.

These observations are consistent with findings of Robison and associates\textsuperscript{29,145,146} that exposure to crystalline NiS, CoS,

### TABLE XIII

**DNA STRANDBREAKS AND CROSMLINKS INDUCED BY METALS**

<table>
<thead>
<tr>
<th>Authors</th>
<th>Source</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zwelling et al.</td>
<td>L-1210 &amp; V79</td>
<td>Cis- and trans-Pt\textsuperscript{II} produce DNA-protein and DNA-interstrand crosslinks</td>
</tr>
<tr>
<td>Fornace et al.</td>
<td>Several cell types</td>
<td>DNA-protein crosslinks induced by Cr\textsuperscript{VI} in cells and by Cr\textsuperscript{III} in isolated nuclei</td>
</tr>
<tr>
<td>Tsakopos et al.</td>
<td>Rat liver &amp; kidney</td>
<td>Cr\textsuperscript{VI} treatment of rats induces DNA-protein crosslinks</td>
</tr>
<tr>
<td>Ciccarelli et al.</td>
<td>Rat kidney</td>
<td>NiCo\textsubscript{3}-treatment of rats induces DNA strandbreaks and DNA-protein crosslinks</td>
</tr>
<tr>
<td>Robison et al.</td>
<td>CHO cells</td>
<td>Soluble Ni\textsuperscript{II}, Cd\textsuperscript{II}, Cr\textsuperscript{VI}, Hg\textsuperscript{II}, and cryst. Ni\textsubscript{3}S\textsubscript{2}, NiS, CoS, CdS, AgS, and CuS cause DNA strandbreaks</td>
</tr>
<tr>
<td>McLean et al.</td>
<td>Human leukocytes</td>
<td>Co\textsuperscript{II}, Cr\textsuperscript{VI}, Sn\textsuperscript{II} pos; Cd\textsuperscript{II}, Mn\textsuperscript{II}, Ni\textsuperscript{II} equivocal; As\textsuperscript{III}, Mn\textsuperscript{II}, and V\textsuperscript{II} neg. for DNA-unwinding</td>
</tr>
<tr>
<td>McLean et al.</td>
<td>CHO cells</td>
<td>Sn\textsuperscript{II} induces strandbreaks; Sn\textsuperscript{IV} does not</td>
</tr>
</tbody>
</table>
CdS, AgS, CuS, and Ni₂S₂ particles produces DNA strandbreaks in cultured Chinese hamster ovary cells. Additions of Ni[II], Hg[II], Cr[VI], or Cd[II] to the culture medium also produced DNA strandbreaks, whereas Mn[II], Zn[II], and Fe[II] did not. McLean et al. used a novel fluorescence technique to measure DNA damage in human leukocytes exposed in vitro to various metal salts; Co[II], Cr[VI], and Sn[II] gave positive results; Ni[II], Cd[II], and Zn[II] gave equivocal results; and As[III], V[II], and Mn[II] gave negative results. McLean et al. reported that exposure of CHO cells to Sn[II] caused extensive DNA damage, as detected by alkaline sucrose gradient analysis; treatment of CHO cells with Sn[IV] produced no DNA damage under the same conditions. Levis and Bianchi cautioned that DNA strand-breaks should not be accepted as prima-facie evidence of direct DNA damage by metal compounds, since strandbreaks can also be produced by indirect, non-specific effects, such as intracellular release of lysosomal nucleases.

DNA Polymerase Infidelity

Research in Loeb's laboratory has advanced understanding of the effects of metal ions on fidelity of DNA synthesis. Tkeshelashvili et al. showed that Cr[III] and Cr[VI] diminish the fidelity by which E. coli DNA polymerase I copies synthetic polynucleotide templates, leading to single-base substitutions. Cr[VI] also decreases the fidelity by which E. coli DNA polymerase I copies φX174 DNA, a natural DNA template. Zakour et al. summarized DNA fidelity assays of 40 metal compounds; indicating that cations of Ag, Be, Cd, Co, Cr, Cu, Mn, Ni, and Pb increase misincorporation of nucleotide bases in the daughter strand of DNA that is synthesized in vitro from polynucleotide templates by microbial DNA polymerases, while Al, As, Ba, Ca, Fe, K, Rb, Mg, Na, Se, Sr, and Zn give negative responses. Pt compounds were not tested. Miyaki et al. confirmed that carcinogenic metal cations cause notable increases in the misincorporation of all four nucleotides by DNA polymerases in vitro, whereas noncarcinogenic metals do not change the fidelity.

Zakour et al. proposed three possible explanations for metal-induced infidelity of DNA replication: (1) altered conformation at the substrate-binding site of DNA polymerase; (2) altered conformation at the catalytically active site of DNA polymerase, possibly involving allosteric transitions produced by interactions of metals with the enzyme at loci distant from the active site; and (3) altered template-base specificity, interfering directly with complementary base-pairing during DNA replication (table XIV). Loeb et al. reported that exposure of DNA to Ni[II] or Cu[II] leads to hydrolysis of purines by cleavage of bonds between the base and deoxyribose, suggesting that the metal ions may bind selectively to the N-7 position. Copying of DNA that contains the resulting apurinic sites may cause mutations, owing to preferential incorporation of purines, particularly deoxyadenosine.

RNA Chain Initiation

Niyogi and coworkers examined the effects of metals on transcription of calf thymus DNA and phage T4 DNA by RNA polymerase from E. coli B under carefully controlled conditions. These studies confirmed previous observations that, at metal ion concentrations that inhibit overall transcription, Be[II], Cd[II], Co[II], Mn[II], Ni[II], and Pb[II] increase RNA chain initiation, whereas Ca[II], Mg[II], Sr[II], Zn[II], Li[I], Na[I], and K[II] decrease RNA chain initiation. The authors speculated that interactions of metal ions with DNA result...
## TABLE XIV

### EFFECTS OF METALS IN CELL-FREE SYSTEMS

<table>
<thead>
<tr>
<th>Authors</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zakour et al. (191,192)</td>
<td>Carcinogenic metal ions decrease fidelity of DNA replication by E. coli DNA polymerase</td>
</tr>
<tr>
<td>Niyogi et al. (121,122)</td>
<td>Carcinogenic metal ions enhance chain initiation by E. coli RNA polymerase</td>
</tr>
<tr>
<td>van de Sande et al. (179)</td>
<td>Ni[II], Co[II], and Mn[II] induce transition of poly(dG-dC) from B to Z helices</td>
</tr>
<tr>
<td>Bourtayre et al. (12)</td>
<td>NiCl₂, NiSO₄, NiCO₃, and Ni₃S₂ induce B to Z transition of DNA</td>
</tr>
<tr>
<td>Eichhorn et al. (44,156)</td>
<td>Co[II]-induced B to Z transition of DNA causes diminished template efficiency for E. coli RNA polymerase</td>
</tr>
</tbody>
</table>

in multiple initiation of new RNA chains, either at the same or at different sites on DNA templates. Loeb and Mildvan discussed the general concordance between the metal ions that decrease the fidelity of DNA polymerase and those that stimulate chain initiation with RNA polymerase; they inferred that a common underlying mechanism (e.g., metal interactions with the DNA template) could account for both phenomena.

### Helical Transition of B-DNA to Z-DNA

Wang et al. demonstrated that 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). The Z-DNA conformation is favored by nucleotide sequences with alternating purines and pyrimidines—especially alternating guanine and cytosine residues. B-DNA and Z-DNA can readily be distinguished by differences in their circular dichroism and phosphorus-NMR spectra. Nordheim et al. found that Z-DNA is a strong immunogen, and that antibodies to Z-DNA do not cross-react with B-DNA. Using immunofluorescence with anti-Z-DNA antibodies, Viegas-Pequignot et al. identified Z-DNA in mammalian metaphase chromosomes. Chemical modifications of DNA and various metal ions influence the susceptibility of double-stranded poly(dG-dC) DNA to undergo the B to Z transformation. Nordheim et al. speculated that carcinogens may act at regulatory regions of DNA to induce imbalance between the B and Z forms of DNA and, thereby, distort normal transcriptional activity. Van de Sande et al. showed that Ni[II], Co[II], or Mn[II] induces the transition of poly(dG-dC) from B to Z helices. Removal of the divalent cation with EDTA produces instantaneous Z to B reversal. Bourtayre et al. studied B to Z conformational transition of double-stranded poly(dG-dC) DNA induced by NiCl₂, NiSO₄, NiCO₃, and Ni₃S₂. In all cases, the nickel compounds induced the B to Z transition at sub-millimolar concentrations; Bourtayre et al. proposed that stabilization of Z-DNA may be in-
volved in the mechanism of nickel carcinogenesis. Eichhorn and associates investigated the ability of metal compounds to induce interconversions among four conformers of poly(dG-dC) DNA; they showed that Co(NH₃)₆Cl₃ first brings about conversion of the B-DNA to Z-DNA, and then to a structure that resembles A-DNA, and finally to the highly compacted ψ-DNA. Most importantly, they found that Z-DNA is less active than B-DNA as a template for E. Coli RNA polymerase.

Conclusions and Research Prospects

Elucidation of relationships between the physical, chemical, and biological properties of metal compounds and their carcinogenic and mutagenic activities has become a major focus of current research on metal carcinogenesis. Experiments with nickel and/or chromium compounds suggest that differences in (a) mass-fraction of metal, (b) solubility in body fluids, and (c) rate of cellular uptake by phagocytosis, carrier-mediated transport, or membrane diffusion may contribute to the marked disparities in carcinogenic and mutagenic activities of these compounds. Further investigations are needed to confirm or refute the attractive hypothesis that intracellular bioavailability of specific metal cations is the crucial factor, and the corollary hypothesis that compounds of a metal are approximately equal in carcinogenic or mutagenic activities when tested at equitoxic dosages.

In vitro experiments that may be related to metal carcinogenesis are summarized in table XV, based upon publications cited in this article and in the author’s previous reviews on this subject. Among the numerous postulated molecular mechanisms for metal carcinogenesis, the following scenarios for metal induction of somatic mutations seem to be the most promising avenues for research:

1. metal cations may bind covalently to DNA, causing strand breakage and excision of specific nucleotide

| Experimental System | Ag | As | Be | Cd | Co | Cr | Cu | Fe | Hg | Mn | Ni | Pb | Pt | Sb | Sn | Tl | Zn |
|---------------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| Mutagenesis and rec-assays in bacteria | ? | x | x | x | x | x | x | x | x | x | x | x | x | x |
| Mutagenesis in mammalian cells | X | X | X | X | X | X | X | x | x | x | x | x | x |
| Chromosomal damage & sister chromatid exchanges | X | X | x | X | x | x | X | x | x | x | x | x | x | x |
| Morphological transformation | X | X | X | X | X | X | X | X | X | X | X | X | x | x |
| DNA strandbreaks, DNA-protein crosslinks | X | X | x | X | X | x | X | x | x | x | x | x | x |
| DNA polymerase infidelity | X | X | x | X | x | X | x | x | x | x | x | x | x |
| RNA strand initiation | X | x | x | x | x | x | x |
| Conversion of B-DNA to Z-DNA | X | X | x | x |

aFor bibliographic citations, readers should consult the text of this article and previous reviews (160-163).

bCertain Cs, Ir, Mo, Os, Rh, Ru, Se, and V compounds have also yielded positive results.
bases, and leading to frame-shift mutations during subsequent repair of DNA damage;

(2) metal cations may form crosslinks between DNA and proteins or between adjacent DNA strands, causing aberrant DNA replication or repair, and disturbing the orderly progression of mitosis;

(3) metal cations may cause transition from B-DNA to Z-DNA, affecting chromatin structure, and expressing normally repressed segments of the genome (e.g., oncogenes);

(4) metal cations may impair the fidelity of DNA replication by altering the conformation of DNA polymerases at the substrate-binding or catalytically active sites, or by modifying template-base specificity by disturbing complementary base-pairing during DNA replication;

(5) metal cations may bind to histones, non-histone nuclear proteins, or nucleolar RNA, influencing chromatin structure and gene expression, perhaps by modifying the phosphorylation of regulatory proteins.

Current knowledge is insufficient to substantiate that one or more of these postulated scenarios is specifically involved in the molecular pathogenesis of carcinogenesis by any particular metal compound. The major advance during the past four years has been the emergence of such hypotheses, which are amenable to experimental test by existing techniques of molecular biology.

Recent research, summarized by Cooper,27 Payne et al.134 and Gilden and Rice,59 shows that normal mammalian cells contain a set of genes ("oncogenes") which are homologous to DNA sequences in oncogenic RNA viruses and to transforming DNA sequences of certain tumor cell lines, as delineated by transfection experiments. Mutation or inappropriate expression of oncogenes has been implicated in neoplastic transformation. For example, Tabin et al.173 and Reddy et al.141 identified a point-mutation in the C-rasH oncogene of tumor cell lines from human bladder carcinomas, and Muschel et al.116 detected the same mutation in leukocytes and normal bladder cells from a patient with bladder carcinoma, raising the possibility that mutation of the C-rasH oncogene confers a predisposition to neoplasia. Moreover, Eva and Aaronson49 showed that tumor cell lines from two of four methylcholanthrene-induced mouse sarcomas contain a transforming gene that is indistinguishable from the Kirsten murine sarcoma virus oncogene, v-kis; Doolittle et al.42 found that v-kis encodes for a protein homologous to a normal gene product, i.e., human platelet-derived growth factor. Based upon such startling evidence, a unifying concept of carcinogenesis has emerged, which holds that the potential for carcinogenesis exists in all cells, and that neoplastic transformation involves mutation or translocation of a cellular oncogene or of a nearby regulatory gene that normally represses the oncogene.150,155,190

At present, nothing is known about relationships between carcinogenic metals and oncogenes. Determining whether metals bind to specific loci in the genome and whether they cause mutation or activation of oncogenes are exciting prospects for research in metal carcinogenesis. Nickel compounds, such as NiS2, are attractive experimental probes for such studies, owing to their exceptional carcinogenic potency and the availability of a beta-emitting radioisotope (63Ni, half-life = 92 years) that is ideally suited for biochemical tracer studies and autoradiography.
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