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

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

Cobalt

Sources of Exposure

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

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

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

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

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

Mutagenic Effects

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

Carcinogenic Effects

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

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

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

Comments and Evaluation

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

Research Needs

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

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

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

Iron

Sources of Exposure

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

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

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

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

Mutagenic Effects

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

Carcinogenic Effects

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

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

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

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

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

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

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

Comment and Evaluation

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

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

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

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

Public Health Implications and Research Needs

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

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

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

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

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

Lead

Sources of Exposure

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

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

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

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

Mutagenic Effects

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

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

Carcinogenic Effects

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

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

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

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

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

Comment and Evaluation

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

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

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

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

Public Health Implications and Research Needs

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

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

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

Manganese

Sources of Exposure

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

The average manganese content of soil is between 600 and 900 mg/kg. Most drinking waters contain less than 100 μg/l.
Cereals may contain between 10 and 100 mg/kg and are the main source of the element. The daily intake in the diet has been estimated at between 2 and 4 mg but values three times as high have been reported.

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

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

Mutagenic Effects

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

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

Carcinogenic Effects

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

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

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

Evaluation

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

Research Needs

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

Mercury

Sources of Exposure

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

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

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

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

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

Mutagenic Effects

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

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

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

and one in chromosome loss following spindle inhibition.

Carcinogenic Effects

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

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

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

Comment and Evaluation

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

Research Needs

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

Platinum

Sources of Exposure

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

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

Biochemical and Toxicological Considerations

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

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

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

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

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

Mutagenic Effects

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

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

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

Antitumor and Carcinogenic Effects

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

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

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

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

Comment and Evaluation

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

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

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

Research Needs

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

Selenium

Sources of Exposure

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

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

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

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

Mutagenic Effects

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

Carcinogenic Effects

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

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

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

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

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

Comment and Evaluation

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

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

Research Needs

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

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

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

Titanium

Sources of Exposure

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

Mutagenic Effects

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

Carcinogenic Effects

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

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

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

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

Comment and Evaluation

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

Conclusion

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

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

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