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Zirconium

An Abnormal Trace Element in Biology

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ABSTRACT

The action of Zirconium (Zr) on biological systems presents an enigma. It is ubiquitous, being present in nature in amounts higher than most trace elements. It is taken up by plants from soil and water and accumulated in certain tissues. The entry into animal systems in vivo is related to the mode of exposure and the concentration in the surrounding environment. Retention is initially in soft tissues and then slowly in the bone. The metal is able to cross the blood brain-barrier and is deposited in the brain and the placental barrier to enter milk. The daily human uptake has been known to be as high as 125 mg. The level of toxicity has been found to be moderately low, both in histological and cytological studies. The toxic effects induced by very high concentrations are nonspecific in nature. Despite the presence and retention in relatively high quantities in biological systems, Zr has not yet been associated with any specific metabolic function. Very little information is available about its interaction with the compounds of the genetical systems, such as nucleic acids. Apparently, the metal is neither an essential nor toxic element in the conventional sense. However, the increasing exposure to this element through its increasing use in new materials and following radioactive fallout, has increased the importance of the study of its effects on living organisms. The tetravalent nature of the ionic state and the high stability of

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the compounds formed are important factors that need to be considered, as also the accumulation of this element in the brain, reminiscent of the relationship between Al^{3+} and Alzheimer's disease.

Index Entries: Zirconium, action of on biological systems, retention, level of toxicity.

INTRODUCTION

The trace element zirconium (Zr), although ubiquitous in the biosphere and present in higher organisms in amounts comparable or higher than that of other essential elements, such as copper, had been neglected as a biologically important metal. More than two decades ago, the possibility of Zr being an essential trace element was postulated by Schroeder and Balassa (*1*) on certain criteria then available, but they also suggested that it might be a natural contaminant with no physiological effects. Since then, considerable information has accumulated on the distribution, uptake, retention, and the modes of action of the compounds of this metal with biological systems. It has been shown to be present in high amounts in forms available to living systems. Interest has increased in the radionuclide of this element (95_{Zr}) after its identification in large amounts in worldwide radioactive fallouts and uptake and retention in plants and animals. In this review, an attempt has been made to assess the biological significance of this metal and its compounds, which show certain unique patterns of behavior.

POSITION IN PERIODIC TABLE AND GENERAL PROPERTIES

Zirconium is a metallic element with the atomic number 40 and mol wt 91.99, and belongs to the group IVB and second transition series of the fifth period of Mendeleyev's periodic table. It was named from Arabic *zargun*, meaning gold color. Discovered in the semiprecious gem zircon, as orthosilicate, by Klaproth in 1789, Zr was isolated as an element by Berzelius in 1824 (*2*).

In its metal form, Zr is hard and resistant to corrosion, heat, and acid. Cationic salts are hydrolysed to form insoluble but stable zirconyl or zirconium oxysalts. ZrO^{2+} ions readily polymerize with increasing pH, allowing extensive olation and oxolation. Zr forms unstable simple cationic salts such as halides and sulfates, and stable anionic zirconates by boiling (*3,4*). It has a high affinity for free PO^{3+} ions, forming insoluble precipitates, which hamper the coprecipitation of other metals like Cd, Cu, Mn, and Pb (*5*). Whether or not it can act as a catalyst at biological temperature is not known (*1*). Some chemical and physical properties are given in Table 1.

Table 1
Chracteristics of Zirconium

Melting point	valency	Electronic configuration	Ionic radius	Coordination number	Stereochemical structure
1855°C	+2 to +4	like inert gas	0.72°	6 and 7 shared by other members of the same periodic group. 8 is the stable form. The coordination tendency increases as R = N R – O – R R – COO– RO OH	Octahedral to tetragonal, pentagonal, bipyramidal to cubic (*6,7*)

USES

Until the advent of atomic energy, Zr had very few uses other than in cosmetics and jewelry. With increasing industrialization it has found application in different industrial processes (Table 2), because of its relative inactivity, low biological toxicity, resistance to high temperature, and capacity to form stable complexes (*8,9*).

DISTRIBUTION

Distribution in Nature

Zirconium behaves like an essential trace metal in the biosphere, although the possibility that it is a natural contaminant with no considerable physiological effects must be considered (*1*). Its mean concentration in rock is 170 ppm, in soil 300 ppm, in marine sediments 132 ppm, and in sea water 4 ppb (*29*). Since carbonates, hydroxides, oxides, and silicates of Zr are insoluble, dissolved salts may be rapidly precipitated from sea water, producing high concentrations in marine sediments. The major minerals are baddeleyite, a form of ZrO_2, and zircon or zirconium orthosilicate ($ZrSiO_4$). It ranks 9 of all elements in abundance on earth's crust (*29*), 32 among all elements in the universe, and 11 among the trace elements (*30*). Some common sources of Zr are given in Table 3.

Distribution Within Organisms

Analysis of water, soil, and vegetation shows that Zr was ubiquitous in the biosphere and was often present in appreciable quantities.

In Plants (Table 4)

In isolated and purified envelopes of *E. coli* K_{12} strains, an intermediate amount of Zr deposition was detected at the polar head group regions of the membranes or along the peptidoglycan layer (*62*). The dinoflagellate *Gymnodinium brevis* of Florida Gulf Coast was seen to contain 0.34 to 3.4 ppm Zr (*63*), but Sargassum from Guanica contained only traces of 95_{Zr} (*64*).

Table 2
Biological and Industrial Uses of Zirconium

Chemical form	Uses	References
Zr-alloy	Electronic industry	
Zr-silicate, Zr-oxychloride	Antiperspirants and toiletries	(*11–13*)
Zr-citrate, Zr-hexacyanoferate	Removes radiostrontium from the human body and from femurs and gastrointestinal tract of rat	(*14,15*)
Zr-complexes	Displaces Pt and induces immunological tolerance against Al and ethanol	(*16–20*)
Zr-complexes	Anticaries substance	(*21–25*)
Zr-in metallic form	Bone and muscle implant material	(*26–28*)

Following radioactive detonation, marine plants are capable of accumulating a large amount of radionuclides. In the western and southern coasts of Puerto Rico, the rate of uptake of 95_{Zr} by the alga *Padina* was five times greater than that of other nuclides (*64*). In alga *Porphyra*, 50% of the accumulated 95_{Zr} was rapidly lost in 6 d (*65*). 95_{Zr} was recorded in green alga *Ulva pertusa* (*48,66*). In mining districts near Timmin and Elliot lakes in Canada, Zr was found to be concentrated by freshwater filamentous algae (*67*). It is probably taken up by marine plants through absorption on the external surface, since it can form strong complexes either in soluble form or colloidal state with biological surfaces. Because of their higher oxidation state, radioisotopes of Zr are found to be tightly bound to the surface of organic debris and plankton (*68,69*). At several locations in Austria, the natural distribution of short lived 95_{Zr} was collected and accumulated by the lichen (*70,71*). In the lichens *Cladonia-cetraria* 95_{Zr} was decreased gradually with time, more rapidly during winter (*72,73*).

The terricolous mosses *Rhytidiadelphus squarrosus, R. triquetrus, Brachythecium mildeanum, B. rivulare, Ciriphyllum piliferum,* and *Plagiommium ellipticum* growing in the parks of the Russian city of St. Petersburg accumulated the element in larger amounts than in a control park located 40 km outside the city (*74*). In Bieszczady (Poland), Zr content was 4.0 mg/kg dry matter of pasture plants (*75*). Concentrations of 95_{Zr} ranged between 10^{-10} to 10^{-9} mg/kg air dry weight in forests of the Arkhangel district of Russia. The maximum amounts occurred in mosses and in litter, while the minimum amounts were found in wood (*76*).

The movement of radioactive Zr from the soil into the plant is greatly influenced by the absorption and desorption processes of the element in the soil. It was seen to be transmitted through the xylem vessels of wheat seedlings germinated in nutrient medium containing radioactive Zr. But when the plants were transferred to the medium after the seedling stage,

Table 3
Distribution of Zirconium in Soil, Water, and Air

Source	Locality	Form	Mean concentration	Reference
Soil	Scotland	Measured in ppm as Zr	700	*31*
	USA		300	
	Spain		300	
	Lancashire, England		270	
	Russian Steppe		300 and 11.96	*32*
	Forests and gardens of Finland		12 and 5.5 ppm	*1*
Soils over granite Rocks	Guatemala Galici	Zircon	Mineral composition was detected, but amount of the individual salt type was not measured	*33–35*
Volcanic ash bed	Cenozoic localities (Kansas, USA)			*36*
Soil	San Pedro Valley (Benson, Arizona, USA)			*37*
Soil and stream sediments	Eastern Bavaria			*38*
Sandstone	Northumberland, England	Garnet and Zircon	Not measured, but soil characteristics were described under electron microscope	*37*
Soil (130,000 to 250,000 years old)	Central California	as Zr		*40*
Soil	Himachal Pradesh, Brahmaputra Valley (India) Near Qarum Lake, Egypt	Zircon	Minerological composition of the soil was examined	*41–43*

(*Continued*)

Table 3 (*Continued*)

Source	Locality	Form	Mean concentration	Reference
Paddy soil	Philipines SriLanka Bangladesh India Indonesia Thailand	as Zr		*44*
Soil	Rongelap Atoll 3 years after nuclear weapon tests in the Pacific	95_{Zr}		*45*
Soil	Balance under ground nuclear detonation on October 30, 1958	95_{Zr}		*46*
Soil	Sedan ejecta			*47*
Soil	Japan seashores			*48*
Sandy soil	Lowland in Northern Germany	Radioactive Zr		*49*
Soil	Western Sweden (following Chernobyl nuclear accident), areas near Chernobyl, the Ukraine	95_{Zr}		*50–52*

Water	440 Lake waters of Maine, USA	as Zr	0.05–22.5 ppb	*53*
	10–15 rivers of USA and Canada	as Zr	0.1 ppb	*54*
Sea Water	Gulf of Mexico	95_{Zr}		*55,56*
	Coastal area of Tokai-Mura Japan after atomospheric nuclear detonation in China (Radiochemical analysis were carried out from 1974-1982)	95_{Zr}		*57*
	Atlantic II and Shaban/ Jean Charcot, Deeps in Red sea	as Zr	Zr:Hf ratio have been analyzed in sediment samples	*58*
Air		as Zr (during open processing of ores)		*59*
		During production of fireproof articles from ZrO_2		*60*
	Thessalonika, Greece	95_{Zr} (after Chernobyl nuclear accident)	$5KBq^{m-2}$	*61*

Table 4
Zirconium in Vegetation of Finland and Vermont (USA) as Related to Soil Types

Plants	Locality	Zr, in ppm in different soil types		
	Finland	Silicic	Ultrabasic	Calcareous
Lichens		110 ± 14	21 ± 4.8	—
Mosses		40 ± 14	9 ± 1.0	23.3.3
Ferns, fronds		14 ± 2.1	14 ± 3.4	17 ± 1.9
Ferns (*Osmunda regalis, Athyrium angustum, Pteridium latiuisculum, Polystichum acrostichoides*	Vermont	45.5 (mean)		
Conifers (evergreens)	Finland	12 ± 2.8	9.8 ± 3.5	6.8 ± 3.0
Shrubs, trees		13 ± 2.7	19 ± 4.5	18 ± 6.9
Herbs		16 ± 1.6	7 ± 1.7	11 ± 2.0
Grasses		22 ± 7.7	12 ± 3.6	—
Trees, deciduous or evergreen (maple, sugar oak, poplar, birch, hemlock, spruce, pine)	Vermont	121.1 (mean)	Not available	

[analyzed by Lounamaa, 1956 (*32*)]

the root system acted as a barrier against the penetration of Zr. Translocation from root to upper parts of the plants was slow (*77*). The accumulation of Zr by different plant systems is affected by time and partly by the organ concerned, but usually not by the presence of other chemicals. Predominant accumulation was recorded in roots and a lesser extent in above ground organs of pea plant, including the grain (*78*). However, plants growing on ejecta from the Sudan thermonuclear cratering detonation in 1962, concentrated significant amounts of 95_{Zr} and its uptake by roots persisted through three years (*47*). Such uptake through roots was reported in plants grown on soils contaminated with radioactive fallout from nuclear weapon tests and with nuclear reactor byproducts (*45,46,68,79,80*). Adsorption by foliage was also recorded. Various amounts of 95_{Zr} have been reported in barley, maize, and alfalfa. Increased soil moisture enhanced its concentration in plants by 1.5 to 3 times (*81*). From silt contaminated with radioactive waste from Sellafield nuclear fuel processing plant, Zr was absorbed chemically and physically in the form of a complex hydrous oxide (*82*).

Ecklemia cava and *Eisenia bicylis* growing in seawater absorbed 95_{Zr} via roots. The concentration and uptake were faster from water than from marine sediment (*57*). The plants *Eichhornia crassipes*, *Lemna minor*, and *Elodea canadensis*, growing in waste water, absorbed Zr in ionic conditions (*83*). In plots of corn fields established at six sites in Michigan and one in Arizona (USA), Zr was one of the major contaminants (1,000 mg/L) and its amount was not affected in the presence of fertilizers or other contaminants (*84*).

Distribution in Animals

Zr is widely distributed in animals, primarily in soft tissues (*85,86*). Distribution in mammalian tissues is shown in Table 5. In animals, studies on Zr metabolism centered mostly on 95_{Zr}, as a product of nuclear fission (*87,88*). It was followed through body fluid from point of entry and deposited mainly in the bone (*89*). Gastrointestinal absorption of simple cationic Zr salts in laboratory animals was negligible (*90,91*). Parenterally administered salts were slowly absorbed from injection sites. The chemical was readily absorbed as citrate or tartarate (*92*). Radioactive Zr was absorbed at a considerably higher rate in suckling rats (*93*), and it also appeared in newborn rat and in milk following exposure of the mother (*94*). Siliceous sponges *Spirastrella cuspidifera* and *Prostylyssa foetida* accumulated 95_{Zr} by preferential uptake (*95*). Accumulation in two sponges *Ircinia strobilina* and *Spheciospongia vesparia* was, however, very small and the excess was ejected into the water passages (*64,95*). Caribou and wolf tissues from the Anaktuvuk Pass region in Alaska also revealed the presence of the fallout 95_{Zr} (*73*). Percutaneous absorption in rodents was Na < Zr < Zn. The comparative permeability of the skin to the compound ZrPT was rabbit < rat < guinea pig (*97*).

The amount of cationic zirconium (Zr^{+4}) increased with time in mitochondrial fraction of liver after administration of Zr as oxalate. Car-

Table 5
Distribution of Zirconium in Tissues of Wild and Domestic Animals and in Human Body (wet weight)

Wild animals (μg/g)		Laboratory animals (μg/g) (no. of samples given in brackets)			Human tissues and fluid (μg/g)		
Deer		Adults rats			Total blood		6.18
Brain	3.31	Heart	(6)	5.72	Liver	(3)	6.28
Hooves	1.99	Liver	(1)	4.70	Lung	(4)	3.46
Fat	3.09	Kidney	(1)	16.02	Spleen	(4)	1.88
Kidney	14.77	Lung	(2)	3.82	Muscle		18.71
Woodchuck		Spleen	(5)	15.39–47.71	Total human body content 420 mg		
Kidney	7.91						
Liver	3.53						
Fox							
Liver	8.93						
Bat (Whole)	5.14						
Raccoon							
Aorta	11.54						

bohydrate chains of sulfated glycoproteins played an important role as the binding substances (*99,100*). Following intravenous injection, Zr was taken up mostly in soft tissue organs, including liver, lung, pancreas, kidney, and thymus gland. As nitrate, Zr had a fairly high uptake rate in cardiac muscle, which decreased with time (*101*). Although Zr could be detected in the brain, the uptake rate was much smaller than that for other organs, which might be due to the blood-brain barrier (*101*).

Retention of 95_{Zr} has been related principally to the mode of exposure. In rat lungs following intratracheal intubation, it was highest 24 h after administration. It decreased after day 7 of the experiment (*102*). One or two years of exposure to Zr^{+4} in drinking water induced the greatest level of increase in kidney and spleen (*86,103*). A relatively high quantity was deposited in liver, spleen, and kidneys (*94*), and retained to a lesser degree in lung (*104*). Injected and ingested Zr-citrate complexes were retained in blood for sometime, transported to other tissues, and then metabolized (*91*). Whole body accidental exposure to 95_{Zr} in dogs and humans showed primary retention in the pulmonary region (*105*). The effects of aerosols containing 95_{Zr}, when inhaled by mice, initially depended on the temperature of formation. At highest temperatures the deposited particles were retained in the lung and later translocated to the skeleton (indicating an excess burden of 95_{Zr}). At lowest temperature, the highest doses were retained by the skeleton, liver, and lung (*106*).

The whole body retention of Zr for suckling rats, previously treated with cortisone acetate, was significantly reduced (*93*).

Amounts of 95_{Zr} increased in tibia up to seven days after administration, to a higher lever than in other organs (*102*). Young rats adsorbed more parenterally injected Zr salts than adult or old animals, and retained them longer in the skeleton because of vigorous metabolism in the bone marrow (*89*). The amount of Zr in bone marrow of suckling rats was approximately four to five times greater, whereas that in most of their soft tissues was almost same (*93*).

Six hours of exposure to Zr led to 0.27% of the metal being deposited in internal organs (*107*). Only a small fraction was absorbed and selectively fixed in the ovaries, and to a lesser degree in lung and bone. In ovary, Zr induced vascular variation (hypervascularization) one month after exposure (*104*).

95_{Zr} hydroxylacetate, administered intravenously to rats, was eliminated from the blood with a half life of about 70 min (*108*). It is generally absorbed very poorly in gastrointestinal tract via oral administration and mostly excreted through the feces. Less than one per cent of the daily intake of Zr of humans was reported to be excreted in the urine (*98*). The way of excretion was suggested to be the hepatobiliary route like most cationic metals, although Zr-citrate complexes retained in kidney were evidently excreted very rapidly (*109*). Of the intubated Zr in lung, 50% were similarly excreted within 63 d (*102*).

The adult rat showed a much steeper and larger initial loss of Zr. The initial rapid loss of the nuclides from sucklings disappeared at around weaning age, followed by considerably slower exponential decrease (*93*). Following contamination of the skin surface of rats by 95_{Zr} oxalate, the elimination half life of Zr was 20% within 6 h and 80% in 10 d (*107*). Nuclides were easily extracted from the liver because they existed in their free forms in the intracellular fluid (*110,111*). The percentage of urinary excretion was 1.06 for nitrate and 3.73 for oxalate 3 h after administration (*107*).

Epidemiological studies are relatively few. In 36 whole enamel samples from premolar teeth of 11-year-old New Zealand children, concentrations of Zr were lower than those of samples from the USA (*112*). In hair samples from 11 Chinese residents of Hong Kong, a relatively high level of Zr was found (*113*). Presence of Zr in human bone in the Beijing (China) area showed the median of log distributions to be 1.5×10^{-5} g/g ash (*114*).

Uptake Rate and Retention of Zirconium by Tumors

In tumors, Zr was observed to be bound mainly with acid mucopolysaccharides. 95_{Zr}, in the mitochondrial fraction, increasing with time after administration. After injecting Zr-oxalate in Ehrlich tumor, a typical autoradiogram showed greater dominance of 95_{Zr} in connective tissue than in viable tumor tissue and in necrotic tumor tissue regardless

Table 6
Zirconium Concentration in Common Foods

Food types	(μg/g)	Food types (μg/g)	
Cereals and grains	2.08	Fruit	0.54
Dairy products	2.67	Nuts	2.31
Meat/Poultry	1.22	Vegetables	1.56
Seafood	0.54	Oils and fats	4.13

of time (*100*). In tumor and liver, 50–60% of 95_{Zr} remained in supernatant after digestion with pronase E. Tumor uptake and accumulation rates of nitrate were more or less similar to oxalate (*115*). Table 6 shows the concentration of this element in some common foods.

INTERACTION WITH OTHER CHEMICALS

Tissues of rodents fed Zr in drinking water for life showed significantly higher copper content in livers of rat than in control (*116*). Distribution of radioactive indium in the body, with other metals like Zr, ensured its accumulation with subsequent introduction (18–28%) into the kidneys as well (*117*).

Injected 95_{Zr} could be removed by 7-aminoalkyline phosphonic acids in white rats. The lowest deposits in the bones occurred in the presence of diethylene and ethylene diamine dimethyl phosphonic acids (*94*). Femoral bone deposition of Zr, however, could not be removed by these chemicals (*104*). In a strain of *Acetobactor methanolicus*, which is able to accumulate large amounts of gluconic acid, up to 45% of Zr can be organoheterotrophically leached (*118*).

EFFECTS ON LIVING ORGANISMS

Plants

Ecological hazards posed by Zr through damage of living plant systems had earlier been considered to be negligible. Although industrial wastes provided Zr in more assimilable forms than those previously available, no known toxic effect of these effluents had been reported up to 1973 (*119*). As sulphate, Zr influenced growth and production of citric acid in *Aspergillus niger* (*120*). The chloride efficiently removes phosphate ions in an aqueous medium, rendering them unavailable for algal uptake (*121*). Growth of phytoplankton was reduced by passive influence of $ZrCl_4$ through inactivation of phosphorus cycling. In a eutrophic farm pond with *Anabaena circinaris*, dramatic differences in phytoplankton production were observed between treated populations with Zr and controls (*122*). When present in large quantities in water, 95_{Zr} decreased

the populations of Oribatei, Collembola, Gamasida, and Acaria, and also reduced pigmentation and size of many organisms (*123*).

Animals

Short term experiments in the laboratory indicate that $ZrCl_4$ removed PO^{3-} efficiently (*121*) but did not affect the benthic macroinvertebrates (*122*). The lethal doses of Zr varied depending on the salt used and water type in the cases of fathead minnows and bluegill sunfish (*124*).

Absorption was also found to be conditioned by the concentration of calcium in the water (*109*). Zr salts are of low toxicity to animals (*125–127*). The mode of administration is a major factor, with parenteral injection leading to greater toxicity than through oral ingestion.

Very few reports are available about physiological or pathological changes due to acute Zr toxicity. In rats, ZrO_2 was not lethal when given orally at up to 10 g/kg body weight. Intraperitoneally, Zr-gluconate was more toxic than citrate (*125*), the lethal doses being 247 and 1170 mg/kg body weight respectively (*16*). Daily doses of 450 mg/kg were tolerated for 8 d (*17*). The oral lethal dose (LD_{50}), ranged from 853 mg/kg body weight for Zr nitrate to 2290 for sodium zirconyl sulfate, expressed as Zr (*128*). After a single oral dose, Zr oxide was not toxic, oxychloride was slightly toxic, and chloride was moderately toxic (*104*).

Growth rate, survival, and longevity in rats and mice were not reversely affected by 5 ppm Zr in the drinking water in life term studies (*86,103*). In female rats, Zr oxychloride (0.23 g/Zr/kg per day) did not influence the growth curve (*104*). Hearts of male rats fed with Zr weighed 14.6% less than that of the control rats, whereas hearts of females weighed 3.5–7.4% more (*103*). This observation has not been repeated in other animals.

Rabbits inhaling Zr lactate aerosol developed pulmonary granulomata (*129*). Following intratracheal intubation, at day 63, black spots were observed in the alveolar wall of the lung (*102*). At certain concentrations, $ZrCl_4$ induced cerebral and pulmonary disorders as well (*104*). The extraordinary tolerance of animals to Zr had earlier been attributed to the presence of the metal in tissues and reasonably large concentrations in foods. The low toxicity of oral administration was related to its insolubility in the intestine or to low pH of intestine that favors olation of Zr salts (*1*). However, intraperitoneal injection of zirconyl sulfate induced considerable toxicity (*8*). Zr had a low toxicity in inhibiting hepatic succinic dehydrogenase (*91,127*).

Male Sprague-Dawley rats, injected intraperitoneally with 1 mg/kg body wt Zr as chloride, did not exhibit any significant toxicity in urine after 24 h (*130*). Following single subcutaneous injection to rats, 95_{Zr} was deposited mainly in the bone and caused radiation injury to the bone marrow (*89*). Intradermal injection as chloride in the external ear of ICL and CBA/J mice induced dysplasia of cartilage (*131*) with the develop-

ment of benign chondromas (*132*). Blanching of older teeth in adult rats was caused by Zr (*103*).

Microscopical, electron microscopic, and electromicrographic studies showed no cytotoxic effect of ZrO_2 on the bone cells surrounding bone cement implants in rabbits (*133*).

Glucose levels are somewhat elevated in serum of fasting and nonfasting Long-Evans strain of female rat. Glycosuria was found in about half of the animals, and the difference from the control was significant at $P < 0.01$ level. Fasting serum chloresterol levels were high in male mice fed with Zr (*134,103*).

Radiological hazards, following gastrointestinal absorption of 95_{Zr}, were twenty times greater than the figures used by the International Commission on Radiological Protection. In a survey of metabolism, the rat fetus was seen to easily receive a dose greater than 1 rem of 95_{Zr} in the first two months of pregnancy (*135*).

Moderate toxicity of Zr reported by some workers might be due to intracellular metabolism. Insolubility of Zr^{4+}, even at the pH present in lysosomes, may not damage the cells (*136*). Macrophages collected after intraperitoneal injection of Zr as oxide showed a decreased level, whereas there as an increase in PMNs observed even 2–3 d after injection (*137*).

Hypersensitivity induced through inhalation of Zr^{4+} aerosols (*138*), though to a moderate extent when compared with other allergens (*136,139*), was of delayed, cell mediated type. It caused allergic granulomatosis, arising as a foreign body reaction without any immunological mechanisms being involved when administered to nonsensitized animals (*1,140,141*). Certain experimental granulomas produced in guineapigs sensitive to Zr may contain varying amounts of rough endoplasmic reticulum (*142*). Intradermal and intraperitoneal injections of Zr salts in CBA/J mice produced local foreign body type granulomas that regularly persisted over eight months. Delayed immune type of epitheloid cell granulomatous hypersensitivity, such as was induced in humans, was not seen (*132*). General structure of the granuloma elicited by *Schistosoma* eggs in rodents was very similar to that of zirconiosis, although the cause remained unknown (*143*).

After inhalation of insoluble Zr salts, acute lung lesions developed due to Zr and Al compounds in hamsters (*144*). Following intradermal injections of 5.0 and 0.5 mg of Zr carbonate and Zr aluminum glycine complex (ZAG) respectively to guineapigs, the site usually contained some macrophages that had ingested crystalline material. ZAGs increased skin thickness 14 d after injection, reaching a maximum at 21 d. the lesions showed granulomas consisting of shredded bundles of intensely basopholic collagen and many giant cells and histocytes that were highly polymorphic, hyperchromatic, occasionally phagocytic, and succeeded by intense fibrosis (*23,145*). Rabbits inoculated intradermally with ZAG and sodium zirconium lactate (NZL) did not show positive skin

reactivity and macrophage inhibitory factor production, but multiple injection of NZL inhibited marginally macrophage migration and skin reactivity (*141*). In vitro, when alveolar microphages from the rabbit were exposed to Zr, lysosomal hydrolases increased significantly in the mitochondrial fraction. Macrophage phagocytosis was observed morphologically in the exposed cell cultures (*146*).

Human Beings—Level of Exposure and Diseases

Human exposure to Zr is not uniform. A large amount of zircon was reported to be consumed in the United States. It could pass both the placental and mammary barriers, and was located in milk taken into polyethylene bottles directly from the udder of a cow. Daily oral intake by humans was reported as 3.5 mg (*1*), remaining fairly constant throughout life. Zr poisoning may occur because of excessive exposure, but industrially, it had not been considered to be a health hazard (*98*).

Certain epidemiological studies indicate different types of toxicity, mainly related to the type of exposure. An admixture of radioactive elements in the dust of nonsoluble Zr-oxides and Zr-silicates, used as raw material for manufacturing fireproof materials, has been reported to cause and intensify the fibrogenic action on lungs (*60*). Among 136 workers in Ceylon exposed to a number of minerals including zircon, six developed nonspecific respiratory dysfunction (*147*). Radiation hazard did not occur below 1.10^{-9} Ci/g of Zr (*148*). In a department engaged in the manufacture of plumbous titanate zirconate, 42 workers complained of occupational dermatitis with hyperhydrosis of hands and tarnishes, 18 of whom reported a "sweet" taste in the mouth and general indisposition. In addition, a number showed an elevated thermal pain sensitivity and electric permeability, along with increased sweating and reduced capillary resistance to the skin (*149*).

Some effects are also found in lungs of workers in the refractory industry, employed for periods of 25–50 yrs in an atmosphere concentrated with radionuclide of the element (*148*). Hand finishers of Zr metal reactor, however, working for 1–17 yrs, showed no definite pathological effects (*150*).

Zr granulomas in humans have been reported after cutaneous exposure to deodorant sticks that contain approximately 0.5% zirconyl sodium lactate (*151,152*), of allergic epithelial origin (*153,154*). Extensive sarcoid-like granulomas of glabrous skin as a result of allergic hypersensitivity to insoluble Zr oxide were also described. These effects resembled those caused by beryllium (*155,156,157*). Hypersensitive cells produced by intradermal injection of diluted Zr lactate were derived from mononuclear cells (*153,158*). A study of the composition of nineteen metals, including Zr, in the brain tissue and cerebrospinal fluid in patients with organic brain diseases showed a direct correlation between the content of

this metal in the brain tissue and CSF. In addition, a relationship was found between the content of individual metals with the amount of water (*159*).

Six patients with dialysis osteomalacia were studied before and after treatment with desferrioxamine. Before treatment, all six had severe osteomalacia with histochemical evidence of metal Zr. After treatment, a decrease of Zr was associated with improvement in clinical, biochemical, radiological, and histological parameters. These observations suggested the possible role of this metal in the pathogenesis of dialysis osteomalacia (*160*).

Effects on Genetical Systems

In general, the cytotoxic effects of Zr were very low. The earliest report is of mild turbagenic action of Zr^{4+} in onion root tips (*161*). In Syrian hamsters, intravenous exposure to 2000 PuO_2-Zr_2 spheres per animal did not induce tumors, even at doses up to 118 nCi/g lung tissue (*162*). However, chromosome aberrations were induced by inhalation of 238 PuO_2 microphores containing ZrO_2 dust. Chromosomal aberrations increased 63 to 138 h after cell cultivation (0.18 and 0.24 aberration/cell, respectively). Mitotic indices were greatly depressed in lung tissue culture when irradiated with 238 PuO_2 dust. Many star clusters arose from alpha tracks of the microspheres, indicating an abundance of microphores that caused continuous irradiation during culture period (*163*). Zr salts stimulated mouse spleen cell proliferation in direct proportion to the dose. Preincubation of splenocytes with Zr sulphate (2–100/μM) assisted lectinmediated lymphocyte proliferation. At certain concentrations Zr salts possibly acted as lymphocyte mitogens and augmented the responsiveness of immune cells, which could explain the characteristic induction of delayed hypersensitivity and production of immunological granulomas by this metal in vivo (*164*). Following the study of 3_H-thymidine incorporation in cultured cells from C57BL mice, the viability of spleen cells treated with $ZrOCl_2$ exhibited similar changes at concentrations between 1–40 μM and decreased between 80–440 μM. No effects were found with ZrO_2 and $ZrSiO_4$ at 1–400 μM. The degree of 3_H-thymidine incorporation was enhanced by 1–20 μM of $ZrOCl_2$, and inhibited at 40–400 μM. The effect of Zr on the mouse spleen cell depends upon the solubility of Zr salts. $ZrOCl_2$ was found to be weakly mitogenic at a narrow concentration between 1–20 μM, the maximum being at about 10 μM (*165*).

Detailed studies on $ZrOCl_2$, administered as a single acute dose in higher plants, indicated that it was a weak turbagen. Soaking of *Pisum sativum* seeds in concentrations ranging between 1 and 10^4 ppm induced a high frequency of spindle disturbance, directly related to the concentration used in the root meristems of the germinating seedlings. The effect decreased with time and was apparently caused by the affinity of Zr^{4+}

for thiol groups in the spindle (*166*). Oral administration of a single dose of the salt in vivo to mice in various concentrations, taken as fractions of the LD_{50} dose, induced clastogenic effects, including breaks, in the bone marrow chromosomes at different phases of the cell cycle. The action was dose-dependent (*167*). Female mice showed an enhanced susceptibility. In human leucocytes in vitro, the degree of clastogenicity was lower and chromosome breaks were relatively few in number. In all three test systems, however, $ZrOCl_2$ was significantly mitogenic in the initial stages (*166*), but zirconocene dichloride, a compound of Zr, did not show any biological activity (*168*) or recognizable antineoplastic properties (*169*).

Reaction with Biological Macromolecules

The only early report of direct Zr involvement was the inhibition of alkaline phosphate in vivo (*170*) and ATPase in vitro by zirconyl chloride (*91*). The next report was of ferroxidase or ceruplasmin inhibition of Zr^{4+} (*171*).

Later investigations have shown that the gelatinous metal hydroxides of Zr^{4+} are capable of forming insoluble complexes with the enzymes chymotrypsin, D-glucose oxidase, and B-D glucosidase trypsin. These complexes are enzymatically fully active, and might also form complexes with amino acids and peptides. All these complexes are considered to be formed by the reoccupation of ligand sites of the metal ions. The metal hydroxide-amino acid complexes could also subsequently bind an enzyme molecule to give a product completely different from the soluble form of the enzyme (*172*).

Metal chelates of Zr with asparagine and glutamine were reported potentiometrically (*173,174*). The basic Cr(III)-Zr(IV) sulfate complex (compound A), isolated previously, reacted with a series of amino acids at moderate to acidic pH values to produce either 1:1 or 2:1 amino acid:compound A complex. The complexes were usually relatively insoluble and electrically neutral. The absorption of proteins on metal (Zr) surfaces was characterized through its surface concentration, optical thickness, refractive index, and relative surface coverage as a function of time. Lysozyme was reported to be absorbed in a two layer structure on Zr, but ovalbumin was absorbed in a smaller amount in a monolayer structure (*175*). Zirconium compounds form coordination complex and stable chelates with bidentate ligands. A 2:1 cationic complex was isolated from reaction of an excess of alpha alanine with compound A (*176*).

Interaction of macromolecules like acid mucopolysaccharides with zirconyl ion was detected by spectrophotometry (*177*) or by Chapman's method (*178*). Substances normally present in the acid mucopolysaccharide preparations such as protein, glycoprotein, and nucleic acid did not interfere with zirconyl ion (*177*).

Cellular nucleic acid showed a blocking reaction with Zr(IV) oxide

chloride, forming a faint color after staining with toluidine blue. This blocking mechanism was suggested to be caused by salt linkage between the phosphoric acid of the nucleic acid and Zr at low pH (*179*). The uptake of 1-histidine (His), 1-lysine,and 1-arginine by Y-Zr-phosphate was studied (*180*).

Rat tail tendon tropocollagen reacts with a basic Cr-Zr sulphate complex (zircryst: $Na_2Ze_3Cr_2(SO_4)$ $^{(OH)}12$, $6H_2O$) in pH 3.0. Additional crosslinks, both intra and intercellular, were induced into the collagen. The zircryst moiety remained essentially intact throughout, resulting in an increase in thermal stability of the protein (*181*). Certain drugs like ampicillin and dopamine formed stable chelates with Zr^{4+} (*182*). $ZrOCl_2$ enhanced direct plaque formation in mouse spleen cells blended with macrophages obtained from mice intraperitoneally injected with Zr-oxychloride for a week. From this result, it is suggested that the suppressed IgM immune response resulted from macrophages activated by the addition of $ZrOCl_2$ (*183*).

SUMMARY

Zirconium is widely distributed in nature and appears in all biological systems, often in appreciable quantities. Different plant systems, from algae, lichens, bryophytes, and ferns, to conifers and pasture plants have been observed to contain Zr, the amount depending upon the level of the element present in the soil or environment. It is widely distributed in animals, mainly in the soft tissues. The Zr content in the adult human body is about 420 mg. The daily human intake is more than Mn and relatively high for trace metals. The human exposure has been further enhanced through radioactive fallout as measured by the incidence of the radionuclide 95_{Zr}, in soil, water, and vegetation. Some common foodstuffs and beverages contain appreciable quantities of Zr.

The mode of uptake and return cycle of the metal in living systems have not been worked out in all cases. In plants, the intake is principally through leaf or root absorption. In animals, the retention and excretion levels depend on the route of administration and the duration of exposure (Fig. 1). The radionuclide 95_{Zr} is found to be concentrated mostly in liver, lung, pancreas, kidney, thymus gland, reproductive organs, and, after prolonged exposure, in bone. Zr is shown to cross the blood-brain barrier and is deposited in brain. In liver, Zr as cation is concentrated in the mitochondria and is mostly bound to acid mucopolysaccharide. Excretion is suggested to take place through the hepatobiliary route like other metal cations.

Ingestion of Zr has been observed to enhance the copper content in rat liver and the accumulation of indium in kidney. Removal of Zr after deposition in the tissues is very difficult because of the stability of the

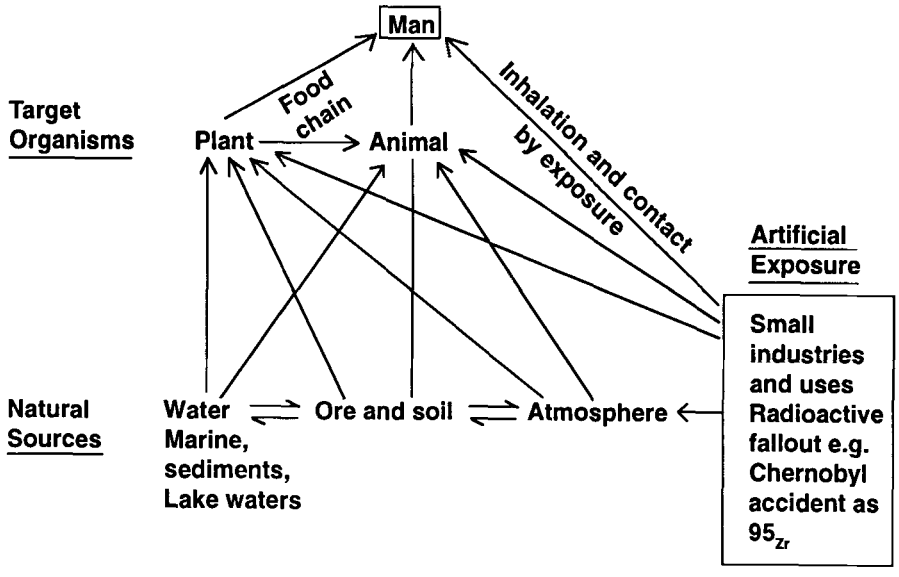

Fig. 1. Possible biogeochemical cycle of Zr from available information.

compounds formed. Only gluconic acid and 7-amino-alkyline phosphoric acids are reported to be able to remove Zr deposits in tissues.

Reports on toxicity of Zr compounds in animals are very few, possibly because of their relatively low affinity for biological macromolecules, low solubility in intestine, or to the low pH of the intestine that favors olation of Zr salts. Human epidemiological studies have suggested the association of exposure to Zr with pulmonary disorders, allergic granulomata formation, and organic disease of brain and osteomalacia. However, these reports have not been confirmed.

Zr salts stimulated the proliferation of cells and were weakly mitogenic, up to a certain level. With higher concentrations, turbagenic, and to some extent clastogenic, effects were induced in higher organisms, following exposure both in vivo and in vitro. Zr has been recorded to inhibit the activity of enzymes like ATPase, alkaline phosphatase, and peroxidase. It also forms metal chelates with amino acids, complexes with enzymes, and binds with acid mucopolysaccharides. As the oxychloride, Zr enhances direct plaque formation, suggesting an immune response. Although it has not yet been shown to enter into the metabolism of any organism, the role of this metal in the biological pathways remains an enigma. However, increasing exposure to this element and reports of deposition in human brain necessitate further investigations on any association with degenerative disorders, as found between aluminum, which has very similar properties, and Alzheimer's disease.

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