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THE PHARMACOLOGY AND TOXICOLOGY OF THE BONE SEEKERS[1]

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INTRODUCTION

Bone as a tissue has been until recently largely neglected by pharmacologists. A major objective of this review is to call attention to the importance of the skeleton in any pharmacological or toxicological consideration. The extreme paucity of information, first as to what drugs go to bone and second as to how bone influences their pharmacological responses, does not reduce the potential importance of this organ system, but instead reflects only the current, inadequate state of our research methodology. A noncolored, nonfluorescent, or nonradioactive bone-seeking substance may go unnoticed or be lost in a distribution-excretion study as "per cent unaccounted for." As a matter of fact, it has only been since isotopes became generally available that great strides in our knowledge of bone metabolism have been made. If nothing else, it is hoped this review will stimulate pharmacologists to pay greater attention to the skeleton.

In this first annual review of the biological effects of the bone seekers, there is a great temptation to present an all-inclusive picture of the many interesting recent studies of the biochemistry of bone, its morphology, normal and abnormal physiology, and its unique importance in radiation biology. Even if such a review were possible within the brief confines of this chapter, however, an exposition of a few modern concepts to serve as an orienting framework of ideas should prove to be a more useful contribution. Such a framework will form a basis against which new information, now accumulating at an astounding rate, may be evaluated. For this reason an encyclopedic or annotated bibliography of scientific papers on bone seekers will not be presented; specific experimental data have been omitted. Also excluded are reports of substances or drugs that act primarily on the skeleton (vitamins and hormones) as well as discussions of the abnormal physiology of normal bone constituents, e.g., calcium, sodium, and magnesium.

Bone seekers as a group comprise a heterogeneous list; by broad definition this includes any substance localizing in the skeleton. Thus several col-

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loidal dyes of large molecular size (lithium carmine and trypan blue) have been shown to stain the organic material of new bone and to persist after decalcification procedures. Alizarin dyes and related anthraquinones also stain new bone by forming calcium lakes. The early studies on bone growth with madder were based on the red color of skeleton laid down during feeding of the dye.

Tetracyclines complex calcium in solution and have been shown to localize in bone by ultraviolet-fluorescence techniques. Although chemotoxicity to bone has not been observed at therapeutic levels, tetracyclines in large doses have been shown experimentally to inhibit embryonic bone development of chicks. Presumably many other substances not now recognized as bone seekers will ultimately be so classed when information on their distribution characteristics becomes available.

It is apparent that an inclusive discussion of the toxicology and pharmacology of bone seekers would be almost impossibly complicated. In this review, emphasis has been placed on two toxicological aspects: deleterious effect in the skeleton and systemic deleterious effects.

DELETERIOUS SKELETAL EFFECTS

A major impetus behind the research interest in the physiology and toxicology of the skeleton was the early recognition that many of the radioactive elements produced by nuclear fission deposited in this tissue. The deleterious effects of extremely minute concentrations of these elements were produced by virtue of their radioactive emanations. Thus, the great toxicological problem involving the skeleton per se is the assessment of the potential hazard to man of long-term low-level exposure to these nuclides.

Basic Physiological Concepts

Skeletal mechanisms.—The dynamic nature of the skeleton is well illustrated by its chemical reactivity. Since a significant portion of total bone is always in rapid equilibrium with blood and extracellular fluid, the importance of bone in any consideration of total body metabolism is understandable. As emphasized by Neuman & Neuman (1, 2) bone is, however, a highly specialized tissue and unique in many respects.

Total skeletal metabolism can be divided into three physiologically distinct compartments: (*a*) area of new bone formation, (*b*) area of bone resorption, and (*c*) stable bone. Children, depending on their age, have bone formation rates in excess of resorption which leads to net skeletal growth. Obviously, areas of bone formation and resorption must be in intimate contact with blood and extracellular fluid, and therefore these areas may be termed the "available skeleton." Stable bone, by definition at least, is not in contact with body fluids and this is the "unavailable skeleton." Incorporation of bone-seeking elements into the available skeleton accounts for the major fraction of skeletally deposited material.

At the microscopic level, turnover in adult compact bone proceeds pri-

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marily through the process of haversian remodelling, i.e., the development of new osteones to replace those lost from resorption cavities through osteoclasis. Initially, these new osteones incorporate mineral at extremely rapid rates, whereas the completeness of mineralization of individual osteones varies with their age. In growing individuals, endochondral bone formation primarily at epiphyseal sites produces a relatively large volume of "available" trabecular bone. Diaphyseal membranous bone formation constitutes another component of the available skeleton. Mineral, including foreign bone-seeking elements, deposited at these sites may be derived in part from that removed from other sites by resorption.

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About one-third of the fat-free dry weight of bone is composed of organic material. Only a small fraction is cells; most of the organic weight is the bone matrix which is impregnated with mineral. The major constituent (95 per cent) of matrix is collagen, similar in chemical composition to that from other connective tissue sources in the body, exhibiting a characteristic major cross-banding at 640 Å intervals. Based on x-ray diffraction and biochemical studies (3, 4), the role of collagen in the induction of biological calcification (nucleation) is becoming increasingly apparent. The remaining fraction of the matrix is the amorphous ground substance, consisting mostly of polymers of glucuronic acid and hexosamines such as chondroitin sulfate and hyaluronic acid. This component of bone has been least characterized and its importance has not been defined.

The major constituent of bone is mineral in the form of hydroxylapatite crystals, highly hydrated and with an extremely large total surface area resulting from their minute size. Thus, surface chemistry assumes paramount importance in bone physiology. The term hydroxylapatite refers to a particular type of crystalline structure, rather than to a specific chemical compound. Although usually represented by the formula $Ca_{10}(PO_4)_6(OH)_2$, the actual composition of bone mineral *in vivo* reflects the chemical composition of the immediate environment in which it was formed or with which it subsequently comes in contact. Depending upon their charge and ionic size, a number of ions may be substituted in certain positions of the hydroxylapatite lattice for Ca, PO_4, or OH, so that normal bone mineral contains varying amounts of Na, Mg, CO_3, and other ions in trace quantities.

Ion-exchange substitution constitutes the major mechanism for skeletal fixation of any bone-seeking element. Upon transport by the blood to the immediate environment of existing bone crystals, a rapid diffusion occurs into the "hydration shells" surrounding the individual crystals. From these hydration jackets, ionic exchange reactions may take place with Ca, PO_4, or OH ions on the crystal surface. For example, calcium exchanges on a 1:1 molar basis with Ca^{45} or Sr^{+2} and on a 2:1 basis for Ra^{+2} or UO_2^{+2}. The rate-limiting factor for this exchange in the case of the alkaline earths (Ca^{45}, Sr, Ra) seems to be the rate of "thermal escape" of calcium from the crystal surface, since these three ions of distinctly different sizes all enter the surface at comparable rates.

Following incorporation into crystal surfaces, certain ions are capable of incorporation into the crystal interior. The process of "recrystallization" is a relatively slow one, and only certain ions of proper ionic dimensions (Sr^{+2}, Ra^{+2}, F^{-}, and probably Pb^{+2}) can participate; others (Na^{+}, CO_3^{-2}, $citrate^{-3}$, Mg^{+2}, and UO_2^{+2}) apparently cannot.

Depending upon the particular substance, skeletally deposited materials soon become fixed by one or more of three processes: (*a*) recrystallization which essentially removes the isotope from surface exchangable sites; (*b*) apposition of new mineral so as to "bury" the ion in the crystal interior; and (*c*) a process termed "diffusion locking." As individual bone crystals grow by accretion of new mineral, these crystals with their highly oriented "hydration shells" attain such close proximity that the diffusion mobility of charged ions within or into the hydration layer is limited. Such ions are effectively excluded from further exchange reactions even though they may reside at surface positions.

Total exclusion of certain bone-seeking isotopes from "stable bone," however, does not occur. Recent histological evidence has shown that in addition to the exchange phenomena described above which require minutes or hours for completion, a process of "slow exchange" takes place (5). This usually requires one to two weeks to become apparent on autoradiographs as a remarkably uniform and diffuse low-intensity distribution throughout the entire bone sample, clearly differentiated from high-intensity "hot spots" (or new osteons) and other areas of new bone formation (i.e., the epiphyses). The exact mechanisms involved in this process are conjectural.

Neuman and co-workers (5) have recently proposed that a local lowering of pH is a mechanism for mineral mobilization. This is a physiological process directly related to parathyroid gland activity, which in turn is regulated through serum ionic calcium levels. Attempts to remove elements deposited in stable bone can only be truly successful through normal resorptive mechanisms. Only if the element were located exclusively on crystal surfaces in the available skeleton could one expect to remove it effectively with a chelator or exchange agent, thus explaining why such agents are efficacious only when administered before, during, or immediately after the exposure to the bone seeker. Since complete decalcification would be necessary to remove substances from stable bone (obviously unfeasible), the experimentally observed poor success of "skeletal detoxifying agents" (7, 8, 9) in late mobilization of skeletally deposited isotopes is understandable.

Blood transport.—The chemical nature of an element is of paramount importance in determining its physiological handling by the body. Obviously the entrant step is greatly dependent upon solubility as well as other factors. Once absorbed, the blood-borne element is transported to sites of deposition or carried to excretory organs for elimination from the body. Hydrolysis, colloid formation, formation of complexes with normally occurring ions or by binding to proteins may affect the behavior of the element. Although

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there are great differences among the various groups of elements, striking similarities exist for elements of a given group. For example, the alkaline earths, calcium (10), strontium (11), and radium (12), are transported in plasma mostly in diffusible (mainly ionic) form. About 30 to 35 per cent of the total serum concentration of these alkaline earths is held as a nondiffusible, protein-bound moiety. Yttrium (13) and plutonium (14), on the other hand, occur in negligible concentration in the diffusible fraction; essentially all that present in blood is in nondiffusible form.

A dynamic equilibrium should exist between the free and protein-bound fractions so that any process which removes the diffusible components effects an instantaneous redistribution. However such an equilibrium probably does not apply in the case of nonfilterable complex colloids, and the importance of these colloids in blood transport and distribution is not well understood.

Excretion.—That portion of any element existing in diffusible form in plasma would be expected to filter through the glomerular membrane. Its appearance in bladder urine, however, would depend upon the relative contributions of reabsorptive and secretory mechanisms acting within the renal tubules. In general, these mechanisms, primarily reabsorptive, operate to conserve physiological ions (e.g., calcium) more efficiently than nonphysiological ions. Such mechanisms lead to "discrimination" against the foreign ion, a fortunate state of affairs especially in the case of Sr^{90}. The kidney discrimination against strontium can be explained in part by the lesser protein binding (leading to more efficient glomerular filtration) and in part by the more efficient tubular reabsorption of calcium. Unfortunately, attempts to improve on the natural renal discrimination against strontium have met with failure. Any process tested which interfered with renal reabsorptive mechanisms affected the physiological ion, calcium, more than the unphysiological ion, strontium (15).

Basic Radiological Concepts

Radiation injury.—Essentially all ionizing radiation, if absorbed, may produce reversible and nonreversible alterations of intracellular molecular structure (16). Even the nonreversible alterations generally do not result in immediately observable damage; rather the effects, in some unknown way, become manifest after a variable latent period or may be such as to produce no recognizable deleterious effects at any time.

Exposure to relatively large doses of externally applied whole-body irradiation (e.g., 300 to 500 r of x-ray or γ-radiation) produces certain characteristic symptoms and signs (17, 18, 19). These may be conveniently divided into: (*a*) acute effects, appearing within an hour after exposure and lasting for 24 to 48 hours, consisting of severe headache, nausea, vomiting, anorexia, irritability, and malaise; (*b*) early effects frequently following a few days of well-being and reaching a maximum in two to three weeks, consisting of bone-marrow depression, erythema, epilation, mucous membrane

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ulceration, fever, and fatigue; and (*c*) late effects frequently requiring years to become manifest as cancer, cataracts, impaired spermatogenesis, genetic mutations, and early senility.

Radiation damage from a bone-seeking, γ-emitting radioisotope differs in no important respect from externally applied radiation (18). Gonads and other soft tissues as well as the bones would absorb the radiation dose. Qualitatively, however, radiation from an internally deposited α- or β-emitting isotope differs in many respects from the more penetrating γ-radiation (20). Because of the relatively short effective range of their emanations, local bone effects from α- or β-emitting bone seekers would far outshadow general somatic and genetic effects. Similarly, bone effects from the α-emitting isotope would be even more localized than those from the β emitter, and the latter would be expected to affect bone marrow to a relatively greater extent.

Another important consideration is the marked nonuniformity (21, 22) of skeletal uptake of all bone-seeking agents, discussed previously. Since radiation effects are proportional to absorbed dose, this nonuniform distribution assumes great importance in evaluating potential radiation hazards and greatly complicates estimations of the precise radiation dose delivered to the skeleton by α and β emitters (23, 24, 25).

A given dose of radiation insufficient to produce acute or early effects may still give rise to a variety of manifestations. The incidence of these late effects decreases as the dose decreases; the lower limit is the natural background radiation from our environment (cosmic rays, soil, etc.). Of great present concern (26, 27, 28) is the assessment of dangers from low levels of radiation which produce an extremely low incidence of observable effects. Of necessity, the preponderant data have been obtained on radiation levels above the levels of interest, i.e., maximum permissible exposure allowed for occupational exposure and radiation dose from Sr^{90} fallout. To extrapolate to the lower dose levels, it should be known whether the dose-response curve is linear or nonlinear and whether there is a threshold or no threshold for the radiation effect (29). Inspection of available experimental data reveals a scatter of points which allow no immediate and simple answer. According to the specific data consulted, lines can be drawn to prove any of the four possibilities, three of which must be wrong for any individual radiation response considered. The threshold concept itself is subject to significantly varying interpretation so that the threshold "to what effect" must be rigorously defined (30).

In assessing the risks of low-level radiation (i.e., for fallout), reasonable conclusions can be reached only if extremely large populations are studied; but it is doubtful whether with the gross criteria now used we will ever be able to demonstrate statistically valid effects on the human population (31). For example, two of the recognized carcinogenic effects associated with large doses of bone-seeking isotopes are leukemia and bone tumors. Granted that the general population has not been exposed to radiation levels

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much above prenuclear age background, we turn to workers in the atomic industry. These in the United States have been estimated at 0.25 per cent of the population, which, with a normal incidence of these tumors would mean only nine individuals per year with bone tumors and 30 with leukemia—a clearly insufficient base on which to pass statistical judgment. The formidable task of directly assessing radiation risk to man is apparent. More sensitive indicators of cellular or functional damage are clearly needed (24, 32).

Radium as a standard.—The paramount importance of radium in assessing radiological hazards of bone-seeking isotopes follows from the fact that a large body of direct clinical observations at a wide range of dose levels have conclusively established that this radionuclide has deleterious effects on the human skeleton (21, 32, 33). The metabolism and skeletal uptake (34) of radium resembles the other alkaline earths, calcium and strontium. Since no such comparable data are available for other radioactive bone seekers, potential hazards must be assessed against the background of the radium experience.

Body burdens above 1.0 μg. radium^{226} are clearly hazardous. With very high Ra doses, effects may become manifest early, as was seen in some dial painters four to six years after exposure, typified by severe anemias and by necrosis of the bones of the jaw. These effects may be clearly differentiated from those which are produced after a longer latent period. In a high percentage of cases, there were roentgenographically detectable bone changes, microscopic and macroscopic necrosis, and bone-tumor development 10 to 20 years after initial exposure. Yet it should be pointed out that some individuals having a present body burden considerably above 1 μg. Ra^{226} (up to 14 μg.) show no detectable evidence of deleterious effects even after several decades of exposure.

In the range 0.5 to 1.0 μg. radium, statistically fewer individuals exhibit toxic effects. The lowest level at which minor roentgenographic changes have been described has been in a patient who after 20 years had a body burden of 0.4 μg. Major skeletal damage in other patients has occurred as low as 0.7 μg., and one case of neoplasia has been found at 0.8 μg. No detectable changes have been recorded below these levels (32).

The radium^{226} maximum body burden was set by an advisory committee to the U. S. National Bureau of Standards at 0.1 μg (0.1 μc.) in 1941 and has remained unchanged although there is not complete agreement on this point. This allowable body burden of Ra^{226} for occupational exposure is a thousand times the usual content of about 0.0001 μg. Ra in the adult (35) derived from natural sources. An important consideration, tending to suggest that this value is a conservative one, is that all available data are based on estimations of the body burden several decades after the presumed initial exposure. The 0.1 μg. "radium standard" is thus a terminal value and extrapolations utilizing one of several formulae are necessary to estimate the much greater original dose. Furthermore, the original dose in many in-

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stances, especially in the dial painters, was not pure Ra^{226} but contained significant quantities of Ra^{228} (mesothorium) with the short half-life of 6.7 years which therefore could not be detected at the time most radium patients were studied. Contamination with mesothorium would greatly increase the predicted toxicity, for not only would the initial dose rate have been much higher, but the relative toxicity of mesothorium has been reported to be much greater than radium. Finally, the 0.1 μg. of Ra^{226} refers to a maximum value reached after 50 years of chronic occupational exposure rather than an initial permissible dose. The total radiation dose delivered through chronic exposure over the 50-year period should be much less. Also a more uniform distribution throughout the entire skeleton would be expected since areas of high concentration in bone as a result of acute administration would be avoided. Thus the 0.1 μg. maximum body burden for occupational exposure may contain a built-in safety factor of two- to fivefold.

On the other hand, it has been pointed out that although there are many data for very low and very high Ra body burdens, few cases with body burdens at or near the recommended maximum body burden of 0.1 μg. have been studied, and the period of observation has not extended in any instance through an entire normal human lifespan. Techniques for detecting early or minor histopathological changes are not available so that the ultimate safety of the Ra^{226} standard has not been unequivocally established.

In any event, the basic method used for estimation of the maximum permissible body burdens for bone seekers other than radium is to calculate the quantity of such an isotope which would deliver to bone a dose in rems equivalent to that delivered by 0.1 μg. Ra^{226}. Even though the organ burden of Ra is assumed, a precise calculation of the exact dose delivered to the skeleton still remains extremely difficult (36). The dose delivered at each step in the decay sequence of radium must be individually calculated and is complicated by the escape of radon (and consequently its daughter elements), which results in considerable loss of energy otherwise absorbed by bone. Such calculations have given a dose rate of 0.06 rads/week (corresponding to 0.56 rems/week) for 0.1 μg. Ra^{226} which is the figure accepted by the International Committee for Radiation Protection (ICRP) (37).

Essentially 96 per cent of the total dose delivered by radioactive decay of Ra^{226} is contributed by α particles (38). To apply such data to β- and γ-emitting bone seekers requires the further application of a correction factor taking into account the well-established observation that equal doses of different kinds of radiation may produce varying biological effects. This has been termed the relative biological effectiveness. Precise estimation of this factor by a physiological end point is not simple but depends on the exact physical and physiological conditions employed. Consequently, the relative biological effectiveness for α particles is based on a purely physical measurement (ion densities along particle track), and a value of 10 has been recommended by the ICRP (37, 38).

After correcting the delivered dose in rads for relative biological effec-

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tiveness to obtain a presumably equivalent dose in rems, equal doses of various bone-seeking isotopes still exhibit significantly different toxicities. Reasons given for this discrepancy include (*a*) less uniform distribution of the istotope in question as compared with radium and (*b*) greater radio sensitivity of its different sites of deposition. To offset this difference, a "relative damage factor" termed "n" was introduced to correct for the presumed increased hazard for a non-γ-emitting bone seeker (37, 38). The value of n is one for Ra and γ emitters and is five for all other isotopes; thus, equal rem doses of Sr^{90} or Pu^{239} appear to be five times as carcinogenic as Ra^{226}, and the n factor takes into account this observed difference. The final calculated value is then called the relative biological effectiveness dose. It should be pointed out that the relative damage factor is being applied by the ICRP in calculations of maximum body burden for all bone seekers by the "radium dose method," but at dose levels far below the experimental levels on which the factor is based.

An alternative method is employed for calculating maximum body burden of bone seekers emitting only γ-radiation where bone itself might not be the critical organ. It is also used for all nonbone-seeking radionuclides. The critique is to limit the weekly dose rate to various organs of the whole body to the limits set by the ICRP for external irradiation [i.e., 0.1 rem/week to gonads and whole body, 0.6 rem/week for skin and thyroid, and 0.3 rem/week to other soft tissues (37)]. Thus the maximum body burden for Sr^{85} which emits only γ-radiation would be based on a 0.3 rem/week since adjacent soft tissues would be irradiated in addition to bone.

All the above calculations assume a uniform distribution of the radioisotope throughout the skeleton. As pointed out under basic physiological concepts, such an assumption cannot be valid because of the markedly different reactivity of the various skeletal compartments. Local dose rates delivered to bone, marrow, or surrounding tissues would depend on the degree of this inhomogeneity of isotope deposition and also on the type and energy of radiation emitted.

Upon consideration of the $Sr^{90} - Y^{90}$ decay process and the geometry of hot spot vs. diffuse labelling, it has been calculated (23, 25) that, although 2 μc. of Sr^{90} would give a dose rate of 11.6 millirad/day if uniform labelling were assumed, the dose rates delivered to marrow and bone range from below 0.5 to perhaps 100 millirad/day, dependent on whether the isotope deposits from chronic (labelling of all mineral) or acute administration (highest labelling in available skeleton). Thus, a certain fraction of bone and marrow would receive doses far above the average dose calculated for uniform deposition.

Translocation of radioactive elements.—The translocation of skeletally deposited isotopes within bone as remodelling occurs has been mentioned. In addition to this transfer of a given isotope from one microscopic site to another site in the same tissue is the possibility of translocation from one site to another as a radioactive element transmutes into another element by

decay. If the decay product is nonradioactive, no problem exists, for the mass of product would be negligible. If, however, the parent element forms a radioactive daughter, then this daughter must be taken into account in all calculations of dose and hazard estimation.

Short-lived daughters constitute a special case. For example Sr^{90} decays with a half-life of 28 years to Y^{90} with a physical half-life of only 64 hours, which is short compared with the time it would require for the body to mobilize the Y^{90} and deposit it in the typical pattern of a rare earth. Release of energy for Y^{90} thus occurs primarily at the original site of Sr^{90} deposition. Similar situations exist for other short-lived daughters.

Radon, a noble gas, is mobilized from bone to expired air relatively rapidly compared to its physical half-life of 3.8 days. Such translocation effectively removes radon and its radioactive daughters from consideration in dose calculations of delivered energy from Ra^{226} decay (38), and measurement of expired radon can be utilized to estimate radium body burden.

The special case where a radioactive daughter may translocate within bone, or between various sites of deposition is illustrated by thorium. Isotopes of this element are members of each of the three natural radioactive decay series: uranium, thorium, and actinium. In each case thorium decays to an isotope of radium, hence the physicochemical and physiological behavior of these thorium daughters is similar to Ra^{226} instead of the parent thorium (39).

Uranium as an Example of a Bone Seeker

No better example of the multifaceted nature of the pharmacology and and toxicology of bone seekers can be presented than that derived from a consideration of uranium. With the advent of nuclear weapons, great impetus was given to a comprehensive physiological and radiological study of this essential fissionable nuclide (40).

Three isotopic forms of uranium (U^{234}, U^{235}, U^{238}, with a relative abundance of 0.006, 0.71 and 99.58 per cent, respectively) occur in nature as relatively insoluble ores. Being α emitters, all are naturally radioactive; but because of differences in half-life, on a weight basis the α activity of U^{234} is approximately 17,000 times, and U^{235} six times, greater than the abundant U^{238}. Similarly, the artificially produced nuclear fuel, U^{233}, has an α activity approximately 27,700 times that of an equal weight of U^{238} (41). Thus, the degree of radiotoxicity depends directly upon whether one is dealing with natural uranium, as in the mining industry, or with the "enriched" uranium utilized for reactors or weapons.

The chemotoxicity of uranium has been well documented (40, 42, 43). The kidney is principal site of uranium injury after it once gains entrance to the circulation. Although several valence forms of uranium exist, the hexavalent, uranyl ion (UO_2^{+2}), is of primary physiological importance in the aqueous environment of the body. This ion complexes readily with various normal organic and inorganic anions which in turn may be diffusible (e.g.,

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principally bicarbonate complex) or nondiffusible (plasma protein complex). As with calcium (10), approximately one-third of the plasma uranium is protein-bound and in dynamic equilibrium with the diffusible forms (42).

Under physiological conditions, normal uranium in complexed form is nontoxic. However, if free uranyl ions are present, combination with surface-located polyphosphate groupings may occur and interfere markedly with normal cellular functions (44).

After intravenous administration of uranium, approximately 60 to 70 per cent appears in the urine during the first 24 hours (45). Thus if an acid urine is being elaborated with concomitant reabsorption of bicarbonate, the filtered uranyl bicarbonate complex dissociates upon reaching the proximal convoluted tubule, allowing free uranyl ion to combine with tubular epithelial cells. Severe renal tubular damage may result. Clearly, then, this bone seeker has a pronounced tissue toxicity quite apart from its potential toxicity to the skeleton. In fact, the calculation of the maximum permissible dose of natural uranium, as well as U^{238}, is based on the kidney chemotoxicity. With more active α-emitting uranium isotopes, U^{233}, U^{234}, U^{235}, the bone becomes the important organ of concern from the radiotoxic standpoint (37). Uranium^{238} can never become a radiological hazard in man because the doses necessary to deposit enough uranium in bone to equal in radiation that delivered by 0.1 μc. of radium would be far in excess of the uranium doses proving lethal by kidney destruction.

These considerations apply to uranium once it has gained access to the circulation. As with all bone seekers, exposure may be to soluble forms, insoluble forms, or both, and three possible portals of entry exist; viz, the gastrointestinal tract, the lung, or the skin. When in a soluble form, rapid absorption may occur through any of these pathways. However, uranium and the majority of radiotoxic bone seekers, with the notable exception of strontium, are poorly absorbed from the gut or unbroken skin in any form (40, 42, 46). If gastrointestinal absorption does not occur, the element whether soluble or insoluble is soon excreted, whereas pulmonary deposits of a poorly absorbed material (insoluble uranium dust, for example) are only slowly mobilized. In this case then, the lung assumes critical importance in assessing the radiotoxicity from the bone seeker (47).

Fallout and Strontium^{90}

Fallout.—The current intense interest in the metabolism of strontium in man is a direct consequence of the development of nuclear weapons. About 200 radioactive isotopes of more than 30 elements are disseminated in a nuclear explosion (48). In addition to particles of the original fissionable material (uranium or plutonium), these consist of radioactive fission fragments of elements occurring primarily in the middle portion of the periodic table. Other isotopes may be created by the strong neutron flux associated with the chain reactions, the most important being the conversion of atmospheric nitrogen to carbon^{14}.

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The radioactive elements formed during the explosion fuse with varying amounts of wholly or partially vaporized debris at the test site resulting in particles of widely different sizes (49). Depending on the force and the location of the explosion, various quantities of the smaller particles may be injected beyond the troposphere (i.e., the air layer extending 35,000 to 55,000 feet above the ground in which the earth's weather conditions are determined) into the stratosphere. Thus with an air burst of a weapon one megaton (TNT equivalent) or greater, it is estimated that 99 per cent of the radioactive debris enters the stratosphere and 1 per cent remains in the troposphere. A water surface or coral surface burst injects only 30 per cent and 20 per cent, respectively, into the stratosphere (50).

Fallout from the explosion may then be immediate (local), intermediate, or delayed (19, 51). Local fallout consists primarily of the large particles reaching the ground within the first few hours. Wind conditions at the time may, however, distribute the debris over a wide area as in the case of the 1954 Marshall Island Tests.

The smaller particles not reaching the stratosphere are rapidly carried away from the test site by air currents. They may circle the earth several times before being carried to the ground, primarily through rainfall, as intermediate fallout. This generally occurs within the first few weeks or months, and the distribution is within the same general latitude as the explosion site.

The small particles injected into the stratosphere constitute the delayed or "world-wide" fallout. These particles only slowly re-enter the troposphere from which they are subsequently carried to the ground through rainfall. The mean residence time of stratospheric debris is now estimated to be considerably less than the older 5 to 10 year estimates. Except in the event of all-out nuclear war where local and intermediate fallout assume great importance, it is the delayed fallout which has created world-wide concern (18, 26, 27).

Measurements one hour after a one megaton detonation indicate that approximately 300,000 megacuries of radioactivity (49) are produced in addition to variable quantities of radioactivity resulting from the associated neutron bombardment. Of the hundreds of different radioisotopes which enter the stratosphere for delayed fallout, most have relatively short radioactive half-lives (minutes, hours, days) or consist of relatively insoluble oxides (i.e., $Pu^{239}O_2$, $Ce^{144}O_2$, $Ru^{106}O_2$, and $Zr^{95}O_2$) and therefore do not constitute an ingestion hazard (52). To constitute a potential hazard, a stratospherically deposited isotope must combine a long radioactive half-life (years) with (*a*) a high fission yield, (*b*) a chemical form premitting its ready incorporation into the food chain leading to man, and (*c*) known toxic effects at certain dosages. Of the isotopes produced through fission reactions, cesium^{137}, carbon^{14}, and strontium^{90} have been, until very recently, the isotopes of primary concern.

Cesium^{137} with a half-life of approximately 30 years, has fission yield of

about 6 per cent from the slow neutron fission of uranium^{235} and, being very similar to potassium, is virtually completely absorbed from the gastrointestinal tract. This isotope and its decay daughter, barium^{137}, emit relatively strong β- and γ-rays and constitute a potential genetic hazard by virtue of its distribution throughout the entire body, including the gonads. Recently, active discussions have taken place concerning the potential genetic and somatic hazards of carbon^{14} to the world's population (53, 54). This isotope is formed through the interaction of fission-produced neutrons on atmospheric nitrogen, i.e., $N^{14} + n \rightarrow C^{14} + p$, and appears as $C^{14}O_2$. Its extremely long half-life (5570 years) and ubiquitous distribution throughout the biosphere, including the genetic material of plants and animals, are the important factors in these considerations. From weapons tested thus far it has been estimated that approximately 0.56 tons of C^{14} have been added to the 44.3 tons existing before testing. The latter appears normally in our biosphere from the constant bombardment of nitrogen by cosmic rays.

Strontium^{90}.—Of primary interest to this discussion, however, is the strontium^{90} contribution to long-term fallout. Strontium^{90} is chemically similar to calcium and satisfies all the hazard criteria enumerated above. In addition, the isotope is concentrated only within bone. Unique characteristics have placed strontium^{90} into a position where it is feared as a serious fallout hazard.

It has been estimated that in the fission of uranium or plutonium approximately 3 to 5 per cent of the fissions result in the formation of a krypton^{90} atom. Its subsequent decay then occurs through the following sequence: krypton^{90}—33 sec.→ rubidium^{90}—27 min.→ strontium^{90}—29 yrs.→ yttrium^{90}—64 hrs. → zirconium^{90} (stable) (55). The importance of this reaction scheme lies in the fact that the actual production of strontium^{90} occurs relatively late, and its precursor, a noble gas, is distributed widely immediately after the explosion. Condensation of radioactive nuclides to test-site debris particles is thought to occur within the first minute (56). Thus local trapping of strontium to prevent stratospheric contamination is extremely difficult. (This is also true of cesium^{137} which is formed through the decay of another noble gas, xenon^{137}.)

Of critical importance in assessing long-term fallout hazard from strontium^{90} are (*a*) the percentage of the total amount of strontium^{90} produced that has already been injected into the stratosphere and the expected contribution from future tests, (*b*) the rate at which this stratospheric inventory reaches the earth's surface, (*c*) the degree of uniformity or nonuniformity of fallout deposition on the ground, (*d*) the extent to which fallout strontium^{90} reaches the internal organs of man, and (*e*) the concentration of strontium^{90} that is toxic to the human organism. All but the last factor enter into the calculations of predicted future levels of strontium^{90} in human beings. Regardless of the accuracy of such predictions, of equal or greater importance is the last-mentioned consideration, i.e., the assessment of the potential hazard of low-level quantities of strontium^{90} in bone.

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During the past few years, lively debates have centered around each of these points with national and international repercussions (26, 27). Four years ago, relatively little data of quantitative nature were available. Assumptions, extrapolations, or estimations were necessary for each of these considerations and, depending upon how optimistic or conservative one wished to be, a safe or serious hazard prediction could be drawn. Since that time more accurate information has become available for strontium^{90} in the first five categories so that the initial sharp controversies have disappeared (57).

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Original estimates of the amount of strontium^{90} injected into the stratosphere were based upon fairly good approximations of the number of megacuries produced per megaton fission yield minus the amount trapped at the site. For bombs exploded by countries other than the United States, the latter figure could only be approximated. The actual stratospheric inventory during a given year was obtained by subtracting the amount of strontium^{90} (estimated by soil analysis) already deposited on the ground. Such calculation gave a value of two to five megacuries in 1959 (19). No further megaton testing has occurred since the Russian series in October 1958. Direct high-altitude balloon and aircraft sampling has since shown that this was an over-estimate, the stratospheric inventory being approximately 0.6 megacuries in the fall of 1959 (58).

Another over-estimate was the length of the mean residence time of stratospheric debris. Whereas five to 10 years was originally given, it now appears that this may actually be less than one year and certainly less than three years. Recent studies have also confirmed Machta's original prediction of nonuniform distribution of global fallout, about two-thirds of the total appearing in the northern hemisphere in addition to uneven distribution within the hemisphere. Seasonal variations in fallout rate have also been noted (57, 58). Of present concern is the significance of geographical "hot spots," i.e., localization of relatively high environmental radioactivity within a particular country or state. These are thought to result from the combination of a high concentration of tropospheric nuclear debris, over a particular geographical area, coincident with heavy rainfall (59). The above-mentioned meterological considerations have led to a renewed interest in the biological hazards of short-lived fission products such as Sr^{89}, Ba^{140}, and I^{131} (60).

Of major concern now is the estimation of the risk of late effects of low-level strontium^{90} irradiation. Strontium is an alkaline earth appearing in the periodic tables just below calcium so that the chemical and physiological behavior of these two elements is similar, though not identical (61 to 64). In most biological processes there is a distinct preference for calcium assimilation and utilization. The extent to which this "discrimination" occurs varies throughout each of the steps in the fallout-plant-animal food chain leading to man (50). Studies designed to determine the magnitude of these factors assumed great importance in the original predictions of future levels

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of strontium^{90} in human bone. Because specific information was not available, the over-all discrimination factor against strontium^{90} (soil to bone) accepted by various investigators varied from four to 20 (57). The weakest link in the chain appeared to be that from soil to plant. In any event, it was necessary to assume in all such calculations that the soil-plant factor remained constant and was related to the concentration of strontium in the soil (expressed in strontium units where one strontium unit equals one "sunshine" unit equals one μμc. Sr^{90} per gram calcium). This in turn depended upon total accumulated fallout. It is now clear that there is no constant soil-plant discrimination. This is because the major source of strontium^{90} in plants is through direct fallout contamination (i.e., absorption through leaves and plant base absorption) so that "dilution" with soil calcium does not occur (65). Thus, fallout rate, rather than accumulated total fallout is the significant consideration, and, as indicated above, this has now been shown to be quite variable. In addition, gastrointestinal uptake of strontium^{90} varies not only with the dietary intake of calcium, but with other materials in the food (almost 99 per cent of the strontium^{90} carried to the ground by rainfall is retained by soil and plants so that drinking water is not considered a source of contamination).

With the availability of the recent data cited above, it now appears that predictions of peak levels of strontium^{90} in ground, diet, and human bone made even within the past one to two years were too high and set too far in the future. The most recent calculations (66) indicate that maximum ground and diet levels from previous tests occurred in 1959 since 4.5 of the estimated 5.1 megacuries injected into the stratosphere had already reached the ground. Maximum strontium^{90} concentrations in human bones were predicted for 1960. For the latter, the values expected in individuals 1, 15, and 26 years old in western culture areas of the northern hemisphere are approximately 2.6, 1.2, and 0.4 μμc. Sr^{90} per gram calcium, respectively. Assuming no further testing, these authors calculated that in 1970 (presumably when equilibrium between biospheric strontium^{90} and the skeletal systems of the human population will be achieved) human bones should average between 0.5 to 1.0 μμc. Sr^{90} per gram calcium. Still to be determined is the degree of statistical variability throughout the world's population and the specific effects of low calcium diets (e.g., rice-diet countries) and "hot spot" areas. In 1959, Langham suggested a spread of five to 10 times the then available predicted mean values (50).

Ample evidence exists in the literature to show that strontium^{90} is carcinogenic in laboratory animals (67, 68). Rough dose-response relationships have been obtained and differences in tumor incidence observed between single intravenous administration and continuous ingestion. A clear dependence of biological effects on dose-rate exists (69). Biological variables such as species, strain, and age of the experimental animals have been found to significantly affect the results (70). While yielding valuable information on the pathological effects of large doses of radiation per se, the major diffi-

culty lies in the extrapolation of such data to low levels of radioactivity in man (67, 71). All animal studies have involved doses of strontium^{90} in the order of 0.1 to 1.0 μc. per gram body weight or intakes of 10 μc. strontium^{90} per gram calcium. As pointed out by Lamerton (72) the mean skeletal dose in those animals developing tumors would be in the order of thousands or tens of thousands of rads. No laboratory information is available on the risk of bone-tumor production from radiation doses less than several hundred rads.

An unresolved question is the extent to which the natural radiation background (cosmic rays, Ra^{226}, K^{40}, C^{14}, etc.) contributes to the normal incidence of tumors and genetic mutations. Opinions vary from "all" to "few." If this incidence were known with any degree of certainty, extrapolations upward for the added dose delivered from fallout would enable precise evaluation of the hazard, since the natural background radiation to man is known with relative accuracy (approximately 0.13 r per year or 7 to 10 r over a 70-year lifespan). The presently recommended maximum body burden of strontium^{90} for the general population set by the ICRP (37) (equivalent to 67 μμc. Sr^{90} per gram calcium) follows from accepting the risk of doubling the natural background dose.

One μμc. Sr^{90} per gram calcium homogeneously distributed delivers a radiation dose rate to bone of 0.003 rads per year (50). Thus, if predictions of Kulp and co-workers (66) prove correct and if no further megaton testing occurs, equilibrium values for strontium^{90} in bone will be far below the ICRP recommended maximum. While it may subsequently be shown that the original concern about the hazard of the long-lived strontium^{90} was unduly great, it did nevertheless stimulate intensive research. Within the space of a few years many important questions were resolved. On the other hand, new problems were brought to light. For example, the newer meterological data have focused renewed attention on the shorter-lived fission products previously not considered as systemic hazards (60).

Members of Inner Transition Series

A large number of bone-seeking elements are members of the two inner transition series of the periodic table. In these elements the outer valence electronic shells are similar, but the inner levels are incompletely filled (73), and one element differs from another only by electrons in one of the inner d or f levels. Thus, only slight differences in physicochemical behavior will become apparent, since the important valance shells are unchanged.

The first such series, with distinguishing electrons in the 4-f orbitals are called the "lanthanides" or rare earths, and the strict electronic definition is commonly broadened to include the very similar yttrium, lanthanum, and lutetium. Elements following radium with distinguishing 5-f orbitals have been classed (73) as members of a second inner transition series and termed "actinides." These include actinium, thorium, uranium, plutonium, americium, curium, and elements subsequent to No. 103.

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Similarities in physicochemical properties between members of each group form a convenient basis on which to discuss the physiological behavior of these elements. Uranium has already been mentioned as a bone seeker ordinarily not capable of gaining access to the circulation except by accidental or intentional injection or by inhalation. Similar situations exist for the other actinides and the lanthanides. However, because of the extensive industrial use of some of these elements and their occurrence in fallout, considerable experimental attention has been directed to these materials.

The rare earths.—A multitude of radioactive isotopes of the rare earth group is produced in fission reactions (26). Their potential danger to man has been said to be small because of their extremely poor absorption (0.01 per cent) upon ingestion (74) and their relatively short half-lives. Total radioactivity remaining 20 days after slow neutron fission of one kg. of U^{235} amounts to about 10 megacuries. About half is present as rare earth isotopes, including lanthanum^{140}, praseodymium^{143}, yttrium^{91}, and neodymium^{147}, with half-lives of the order of several days or weeks. Essentially none of the radioactivity remaining two decades later would be as any of these isotopes (26). It must be pointed out, however, that several of the longer-lived rare earth isotopes could be predicted to contribute a significant fraction to the 10,000 curies still remaining at 20 years. Promethium^{147} with a 2.6-year half-life and samarium^{151} with a 98-year half-life would contribute 3.4 per cent and 2.6 per cent, respectively, compared with the 48 per cent attributable to $Sr^{90} - Y^{90}$. All the rare earths deposit in the skeleton, the heavier members of the series, e.g., thulium^{170}, depositing to a greater extent (75). The skeletal distribution pattern is distinctly different from that of the alkaline earths. Earlier interpretations of an organic matrix distribution were incorrect, for it is now known that yttrium *in vitro* does not bind bone matrix but selectively deposits on bone mineral or hydroxylapatite. The physicochemical solution behavior of yttrium has recently been utilized to explain its preferential deposition in highly calcified nongrowing resorption cavities rather than actively calcifying sites (6).

In neutral solutions, yttrium, unlike the soluble and largely ionic alkaline earths has a strong tendency to hydrolyze and form radiocolloids (13). Furthermore, protein binding of yttrium in serum renders it practically nonultrafilterable (0.5 per cent). Under conditions of either lower pH or increased citrate concentration, or both, yttrium is rendered much more diffusible, precisely the conditions postulated for resorbing bone sites mentioned under basic physiological concepts. Mineral exchange reactions then proceed in the usual way. By inference, other rare earths with similar physicochemical properties would also be expected to deposit at resorbing sites where these unique conditions of pH, complexing ion concentrations, or both, appear to exist.

Plutonium.—Plutonium^{239} is an α- and γ-emitting isotope with a physical half-life of 24,400 years. Since it is one of the primary fissionable fuel elements, plutonium^{239} is produced in large quantities from U^{238} for use in

nuclear reactors and weapons and thus constitutes a potential industrial hazard (76). Although absorbed only 0.003 per cent from the gastrointestinal tract (77), 1 to 10 per cent of an inhaled dose may be absorbed depending on the physicochemical properties and size of the particles. Absorption through skin and accidental punctures also may introduce plutonium into the body.

Absorbed plutonium is transported in the blood primarily as nondiffusible complexes (14) with serum globulins or as colloids, from whence it is rapidly fixed by skeleton and liver. If PuO_2^{+2} complexed with citrate or versenate is administered, skeletal deposition is much greater than liver (70 per cent vs. 15 per cent) whereas uncomplexed Pu^{+3} or Pu^{+4} initially deposits more or less equally between the two organs. Complex colloid formation at physiological pH accounts for the greater reticuloendothelial system fixation in the latter instance.

Microscopically, plutonium fixation by the skeleton is highly localized (78) in a nonuniform manner on endosteal and periosteal bone surfaces and in regions of trabecular bone. Unlike radium, this distribution does not become as diffuse with time.

Although the specific activity of Pu^{239} (0.064 μc./μg.) is lower than that of Ra^{226}, the maximum permissible body burden of 0.04 μc. (37) is lower than that for Ra^{226} because of higher α energy ($E\alpha$ equals 5.15 Mev) and apparently greater radiotoxicity mentioned previously (n equals 5) resulting from the highly localized distribution.

Excretion from skeletal deposition sites is extremely slow with a half-time of about 200 years. In man and dog the urinary excretion is of greater magnitude than via the feces.

Thorium.—Until recently, the most widely used radioactive element in the medical field was the actinide, thorium. Thorium dioxide (Thorotrast) was administered intravenously as a radio-opaque x-ray contrast medium and, being an insoluble colloid, was fixed by the reticulo endothelial system organs: liver, spleen, and bone marrow. Very little translocation of radium daughters from thorotrast was observed (79).

Present interest in thorium revolves around its importance as a nuclear fuel. Although not fissionable itself, Th^{232}, which comprises essentially all of the naturally occurring element is a fertile material like U^{238} and is converted by slow neutrons in a "breeder" reactor to U^{233}, which is fissionable. If intravenously administered as the soluble quadrivalent citrate complex, thorium is bound by the skeletal system (80 per cent) in a very firm manner similar to plutonium (80). Radium daughter isotopes formed by decay, i.e., mesothorium I (Ra^{228}), are handled much like intravenously injected radium, with considerable urinary excretion and deposition in the available skeleton (39). The radioactive hazards from natural thorium include Th^{228}, the radioactive granddaughter of Th^{232}, with a comparatively short half-life of 1.9 years. Thus, the ICRP maximum permissible body burden for natural thorium is only 0.01μc. compared with 0.04 μc for Th^{232} (37).

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DELETERIOUS SYSTEMIC EFFECTS

The remainder of this review has quite a different approach. Attention is limited to three bone seekers—fluoride, beryllium, and lead—which have important systemic effects: Fluoride is selected for its extraordinary public health importance; beryllium because its unmatched metallurgical properties are coupled with insidious and mystifyingly supertoxic properties; and lead to point up a major problem in the toxicology of the future. For each element the effects on the skeletal system are not important or not stressed.

Fluoride

In this country, fluoridated water is now consumed by communities whose total populations exceed 30,000,000. Despite the wide acceptance there still remain groups including a few medical and dental practitioners who oppose this practice for a variety of reasons. Because a great deal of information is available (some 4000 papers on the biological effects of fluorides have been published since 1935) and because the physician and dentist occupy strategic positions in this community program, a brief summary of the pharmacology and toxicology of the inorganic fluorides is presented.

Practically all foods contain traces of fluoride; the average individual probably ingests 0.5 to 1.5 mg. F/day of food-borne inorganic[3] fluoride (81). Relatively insoluble inorganic fluorides, such as, calcium fluoride or bone meal, are understandably less efficient fluoride sources than the completely absorbed soluble fluorides, such as sodium fluoride (82). Fluoride dissolved in a hard water is just as available as the same concentration of fluoride in a soft water. It is none the less true that calcium, like aluminum, and some other substances tend to slow down and reduce the absorption of fluoride into the body. Thus, fluoride in milk is slightly less available and more slowly absorbed than the same amount of fluoride in water (83). Industrial exposures to fluoride-containing dusts serve as sources of fluoride (84, 85).

Regardless of the combination in which inorganic fluoride enters the gastrointestinal tract or the lungs, only fluoride ion is absorbed into the circulation. Fluorides are rapidly distributed through the extracellular fluid and probably through all body water in a pattern nearly identical with that of chloride (86 to 90). In a previously unexposed individual, the distribution pattern is simple: about half the fluoride is promptly deposited in the skeleton, about half promptly excreted in the urine. No soft tissue stores fluoride. Salivary fluoride concentrations are low, approximately equal to those in blood. Milk contains only traces of fluoride. Fluoride crosses the placenta

[3] The biological effects of the organic fluorides (found in certain South African plants; synthetic products) are not considered in this chapter. Their actions are of considerable theoretical and practical interest. The reader is referred to *Toxic Aliphatic Fluorine Compounds* by Pattison (92); to *Phosphorous and Fluorine* by Saunders (93); and to the current clinical literature for information about compounds in clinical use, such as, fluorosteroids, and fluorinated agents used in advanced neoplastic disease.

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and the fetal blood fluoride concentration is approximately equal to that of the maternal blood (91).

From the lengthy list of real or purported effects of fluorides that can be culled from the literature, a few are selected that have been studied in sufficient detail so that quantitative information is available (Table I). It is important to note that the time during which the fluoride effect occurs is almost as important as the amount of fluoride.

Vegetation damage in the vicinity of industrial plants from gaseous or dust exposures to fluorides has long been known. Gladiolus, for example, and peaches are among the susceptible species; air concentrations as low as a few parts per billion cause detectable injury.

First identified by the reduction in experimental rat caries (94, 95), Dean's (96) classical studies of 12 to 14-year-old children in 23 American communities established the relation between the fluoride content of the drinking water and dental caries reduction. With little or no fluoride in the water, the average child had six to nine decayed, missing, or filled (DMF) permanent teeth; with 1 p.p.m. F or more in the water, the average child had three or four DMF permanent teeth. Since 1945, studies (of which the most carefully documented is that in Newburgh-Kingston) have shown conclusively that supplementing the fluoride content of a community water

TABLE I

EFFECTS OF FLUORIDE

Effect	*Amount or Concentration*	*Time*
Vegetation damage	2 p.p.b. in air	not critical
Decreased dental caries	1 p.p.m. ± in water	first 8 yrs. of life and later
Increase in mottled enamel	2 to 8 p.p.m. or more in water	first 8 to 12 yrs. of life
Absence of osteosclerosis	< 5 mg./l. of urine	5 to 10 yrs.
Osteosclerosis in 10 per cent of population	8 p.p.m. in water	lifetime
Crippling fluorosis	more than 20 mg./day in air	10 to 20 years
Weight loss, cattle	30 to 40 p.p.m. in ration	2 to 4 years
Thyroid, altered structure or function, animals	> 50 p.p.m. in ration or water	up to 4 yrs.
Growth depression, animals	> 100 p.p.m. in ration or water	up to 4 yrs.
Kidney, altered structure or function, animals	> 100 p.p.m. in water	6 months
Acute, lethal dose, man	2500 to 5000 mg. F	death in 2 to 4 hrs.

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supply to raise the concentration to about 1 p.p.m. produces mathematically equivalent reductions in caries incidence in children to those in communities where the water is naturally fluoridated (97). The available evidence indicates that benefits continue into middle age (98). The mechanism by which caries is reduced is not understood. A reasonable hypothesis is based on two factors. (*a*) Fluorapatite is considerably less soluble than hydroxylapatite, the normal bone mineral. The surface layer of enamel contains more fluoride than the internal part of the enamel, hence the defenses of the tooth are strengthened. (*b*) Fluoride inhibits acid production by certain bacteria. Perhaps those bacteria lying in immediate contact with the tooth surface and forming acid find themselves in a milieu of markedly elevated local fluoride concentration; in this way the attacking forces are weakened. The safety of water fluoridation is demonstrated by the remarkable pediatric study in Newburgh-Kingston in which no differences in the children's health in the two cities could be detected. One of the principal guarantees of the safety of water fluoridation is the large number of people (over 3,000,000 in the United States) who consume naturally fluoridated water and have done so for many years. No obvious interference with health is known; regrettably, only fragmentary epidemiological evidence sustains this clinical opinion.

The most delicate untoward effect produced in human is mottled enamel. When enamel is forming in the tooth bud the specialized cells (ameloblasts) can be injured by a variety of conditions, such as vitamin deficiency, disease, mechanical injury, or by chemicals, e.g., fluoride. Failure of the proper function of the ameloblasts causes metaplasia or hypoplasic changes which, when they involve the enamel surface, are described as mottled enamel. In mild degree, this is a "normal" occurrence. Surveys have shown that about one of five individuals reared on drinking water containing no fluoride exhibits some white spots of the enamel surface classed as mottled enamel. Note that this is a developmental defect and can only occur during the first eight to 12 years of life when enamel is being formed. Excess fluorides ingested thereafter cannot produce mottling. Teeth of persons drinking water containing about 1 p.p.m. have the same incidence of mottling as is found when the water contains essentially no fluoride. The term "mottled enamel" connotes, however, an unesthetic staining; with increasing concentrations of fluoride, increasing numbers of children exhibit milky areas, brown spots, and even irregularities of the surfaces (pits and grooves). Perhaps one child in a thousand, for example, drinking water containing 2 p.p.m. fluoride exhibits a brown spot, and when water contains about 4 p.p.m. fluoride, about one child in three exhibits some stained surfaces on some teeth. The term mottled enamel thus is used to describe both the "normal" appearance when the drinking water contains little or no fluoride and the disfiguring staining when water contains large excesses of fluoride. For water fluoridation, the important point is that fluoride at 1 p.p.m. does not alter the normal appearance of the teeth.

With continuing exposures to concentrations of fluoride 8 p.p.m. and higher hypercalcification of the skeleton (osteosclerosis) appears. Whether this reveals an increased calcification tendency or a decreased osteoclastic removal of bone is not known. The first bones to show this effect are frequently the pelvic vertebrae. Osteosclerosis is asymptomatic and no interference with the normal functions of the skeleton either structurally or as a mineral reservoir have been detected. A diligent search has failed to reveal any connection between fluoride and arthritis (99). Demonstrable osteosclerosis in man or animal bone does not develop until the fluoride concentration exceeds a limit of about 5000 p.p.m. (100, 101). Osteosclerosis does not develop in industrial workmen if their urine contains less than 5 mg. F/1. Such a concentration must be close to the noneffective limit; about 10 per cent of the residents of Bartlett, Texas, who drank water containing 8 p.p.m. fluoride showed osteosclerosis (102).

A rare industrial disorder called crippling fluorosis was characterized in the classic study of Roholm (103). Excessive exposures, certainly to more than 20 mg. fluoride a day and perhaps to 80 or 100 mg./day or more, will produce in 10 to 20 years the signs and symptoms of this disease identified by (*a*) osteosclerosis (and also loci of hypocalcification), (*b*) calcification of the broad ligaments, e.g., those along the spinous processes, which produces "poker back," (*c*) stiff joints, and (*d*) outgrowths from the surfaces of bone called exostoses. This slowly progressive disease should never be seen in the future. Excellent methods are available for the analysis of urinary fluoride which will give evidence of excessive fluoride exposure that might ultimately lead to crippling fluorosis. The overexposure can be quantitatively identified and industrial hygiene measures instituted to prevent the progress of the disease.

Growth depression appears in young animals of a number of species when diets or water contain 100 p.p.m. F or more (104). Retardation of growth has never been reported in the human. Somewhat lesser amounts of fluoride in the rations of animals produce weight loss, for example, in cattle fed 30 to 40 p.p.m. in the ration for three or four years, evidence of debility appeared.

Because fluoride is one of the halogens, and because large doses of fluoride given to experimental animals in a century-old experiment produced swellings in the neck, questions of a relation between fluoride and the thyroid have been repeatedly raised. Detectable amounts of radiofluorine (F^{18}) are found in the thyroid after an intravenous dose; however, the concentrations are parallel to those in the blood and uniformly less than the blood concentrations. Fluoride is not stored in the thyroid; the normal thyroid like other soft tissues contains only a few p.p.m. fluoride. Large doses of fluoride can produce changes in the structure and in the function of the thyroid; more than 50 p.p.m. in the food or water are required (101). A determined effort has been made to find any relation (*a*) between fluoride and goiter, or (*b*) between the severity of fluorosis and iodine deficiency in regions where

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this deficiency is common. No relations have been discovered despite careful study.

Fluoride is excreted from the body almost entirely via the kidney. A small percentage is excreted in the gut via the feces. The urinary excretion is rapid. The clearance of fluoride is greater than that of chloride, not from more rapid filtration but because fluoride resorption is conspicuously less efficient than chloride. With a normal water load, fluoride resorption may be only 92 per cent as contrasted with greater than 99 per cent for chloride. The very large volume of blood passing through the kidney and the reduced efficiency of resorption accounts for the very rapid excretion of fluoride (105, 106). In advanced kidney disease with reduced urinary function, fluoride is retained longer in the body; greater percentages of the daily intake are consequently deposited in the skeleton (107). Large doses of fluoride, e.g., 100 p.p.m. in the water or more (108), have produced in experimental animals alterations in structure and function of the kidney. The question raised by Ramseyer *et al.* (109), whether 1 to 10 p.p.m. of fluoride in the drinking water produced kidney pathology in a few rats, has been resolved by a more recent study from the same laboratory. Larger groups of rats after long exposure were examined by pathologists independently in three laboratories. Opinions were unanimous that drinking water containing 1 to 10 p.p.m. of fluoride produced no kidney injury. The renal pathology described by Ramseyer *et al.* was the typical kidney changes of infection and old age.

Large doses of fluoride taken orally can produce death. The wide use of sodium fluoride as a household insecticide for many years and the innocuous appearance of the white powder so easily mistaken for powdered sugar, starch, or baking powder have led to many tragic accidents. The lethal dose in the adult human is probably 2500 to 5000 mg. of fluoride (equivalent to 5 to 10 gm. of sodium fluoride). Acute poisoning follows a very rapid course: nausea and vomiting, weakness and collapse, with evidence of circulatory failure, and death in two to four hours. Prompt antidotal procedures are useful and may be lifesaving (110). Because fluoride inhibits the action of many enzymes when the concentrations are sufficiently elevated, this is probably an enzymatic metabolic death. The precise mechanisms are not known.

From a public health standpoint, water fluoridation constitutes a new development. Here a public utility is being employed to treat and improve the health, particularly of the young. Courts have repeatedly held that water fluoridation contributes to the health of young people and is in fact a public health measure. Pleas that water fluoridation violates the constitutional guarantees of religious freedom for those who object to the use of medicines, or violates the constitutional freedom of choice because people are compelled to drink fluoridated water whether they want to or not, have been answered by the general proposition that people may be required to do or not to do certain things for the public good.

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Beryllium

A surging interest in the use of beryllium as a construction material in space vehicles and perhaps as a fuel focuses increasing attention on the physiological properties of beryllium. The toxicology of beryllium in recent years has a turbulent history. A very active debate on the question, "Is beryllium toxic?" preceded the identification of the first cases of acute beryllium poisoning (pneumonitis). How such apparently infinitesimal traces of beryllium (in the air or in the tissues) could be responsible for certain cases of illness still occasions argument. Today, however, there is no doubt that beryllium is a dangerously poisonous element (111). In human patients acute pneumonitis, chronic granulomatosis often with widespread pulmonary involvement, subcutaneous granulomas, and contact dermatitis have been attributed to beryllium exposures. In experimental animals, beryllium causes cancer (lung cancer in rats; bone cancer in rabbits) and stimulates a marked hypercalcification of bone. Whether beryllium poisoning is a systemic or localized disease, whether criteria of allergy are satisfied, or whether there is a dose-response relation are fundamental and undecided points (112). At the heart of the immediate problem lies the example of the Atomic Energy Commission which set a maximal allowable concentration of 2 μg. of beryllium per cubic meter of air, a widely accepted industrial hygiene standard. After 10 years experience with this concentration as a "target figure," no cases of beryllium poisoning have appeared. This constitutes no guarantee that none will appear; as much as 16 years lag has been recorded between exposure and the development of the disabling and frequently fatal chronic granulomatosis. Enforcing rigidly maximum allowable concentration of 2 μg. Be/m.^{3} would be expensive, in some cases too expensive to tolerate. The problem is whether this figure is too stringent and overly protective.

Lead

Interest in lead poisoning continues to be high, both as a result of industrial exposure (witness the steady flow of papers from European and Russian literature) and as a childhood disease in which diagnosis is frequently difficult (113). Diagnostic procedures involving (*a*) the elevation of serum transaminase (114), (*b*) increases in the urinary (115, 116) or in the fecal excretion (117) of lead following administration of ethylenediaminetetraacetic acid tetrasodium salt (EDTA), (*c*) studies of urinary porphyrin mixtures (118), or (*d*) the elevation of urinary amino levulinic acid levels (119) may offer assistance in clarifying a puzzling picture. Studies of the mechanisms of lead effects, such as those on red blood cells by Clarkson & Kench (120) or on heme synthesis by Tishkoff *et al.* (119), may ultimately lead to better diagnostic procedures. In an effort to mobilize the lead in the soft tissues, the time-honored practices of inducing systemic acidosis or of administering parathyroid hormone have been supplanted by such drugs as EDTA or penicillamine (121) or perhaps by 2,3-dimercapto-

propanol (122). The importance of evaluating the renal status before treatment has been stressed by Fried *et al.* (123).

The requirements in the space age to provide safe living conditions for men who will breathe atmospheres 24 hours a day, seven days a week, rather than the traditional 40-hour work week, have opened a new chapter in the study of lead. A reduction of the maximum allowable concentration to one-fourth (40/168) the present industrial standard will be unacceptable on the basis of human metabolism studies by Kehoe and co-workers (124). With intermittent oral administration of lead (simulating a 40-hour week), the lead content of the body increases to an apparent maximum. In contrast, with daily exposures to abnormal quantities, lead has accumulated in the body in a linear fashion for a period of five years. This dramatically emphasizes the need to reinvestigate the problem of controlling lead poisoning to guarantee safe concentrations for continuous exposures. This same uncertainty may be found for other substances, and as a result revolutionary new industrial hygiene practices may well emerge.

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