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Metabolism and Possible Health Effects of Aluminum

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Literature regarding the biochemistry of aluminum and eight similar ions is reviewed. Close and hitherto unknown similarities were found. A hypothetical model is presented for the metabolism, based on documented direct observations of Al^{3+} and analogies from other ions. Main characteristics are low intestinal absorption, rapid urinary excretion, and slow tissue uptake, mostly in skeleton and reticuloendothelial cells. Intracellular Al^{3+} is probably first confined in the lysosomes but then slowly accumulates in the cell nucleus and chromatin. Large, long-lived cells, e.g., neurons, may be the most liable to this accumulation. In heterochromatin, Al^{3+} levels can be found comparable to those used in leather tannage. It is proposed that an accumulation may take place at a subcellular level without any significant increase in the corresponding tissue concentration. The possible effects of this accumulation are discussed.

As Al^{3+} is neurotoxic, the brain metabolism is most interesting. The normal and the lethally toxic brain levels of Al^{3+} are well documented and differ only by a factor of 3–10. The normal brain uptake of Al^{3+} is estimated from data on intestinal uptake of Al^{3+} and brain uptake of radionuclides of similar ions administered intravenously. The uptake is very slow, 1 mg in 36 years, and is consistent with an assumption that Al^{3+} taken up by the brain cannot be eliminated and is therefore accumulated.

The possibility that Al^{3+} may cause or contribute to some specific diseases, most of them related to aging, is discussed with the proposed metabolic picture in mind.

Biochemistry and Metabolism of Al^{3+} and Similar Ions: A Review

Introduction

Aluminum (Al) is present in very small amounts in living organisms but is abundant in the environment. In no case has Al^{3+} been shown to have a definite biological function. Taken together, this suggests that Al^{3+} possesses properties incompatible with fundamental life processes. Despite this, Al^{3+} has generally been regarded as virtually biologically inert and the interest shown for its biochemistry and metabolism has been very limited. However, during recent years, an increasing number of toxic effects have been established. Interest in Al^{3+} has therefore increased, but many basic questions still remain unanswered.

An ion like Al^{3+} is easily bound to many substances and structures in the organisms. Therefore, its metabolism is determined by its affinity to each of the ligands and by their relative amounts and metabolisms. The ligands are often nonspecific and can bind other metal ions having similar properties and this probably applies especially if the ions lack biological functions and, as a consequence, no specific Al^{3+} ligands have evolved. Hence, chemically similar ions might have a very similar metabolism. If so, the lack of knowledge regarding Al^{3+} could be provisionally substituted with data for other similar ions. The following review is an attempt to summarize the current literature concerning the biochemistry and metabolism of Al^{3+} and similar ions and, if possible by comparison, to achieve a more detailed picture of the behavior of Al^{3+} in the body.

General Properties. In nature, Al exists only in the oxidation state Al(III). The ionic radius is small, only 0.51 Å, due to the ion's strong electric charge. The high charge and small size give Al^{3+} a strong polarizing effect on adjacent atoms. Therefore, in aqueous solutions the ion protolyzes part of the water envelope and forms hydroxo complexes. As a result the solution becomes acidic. In inorganic chemistry this is called hydrolysis. If the solution is neutralized, the hydrolysis continues, and dimers and polynuclear complexes are formed. The complexes gradually increase and loose ring structures of Al hydroxide arise. A white precipitate is then formed, which is initially easily soluble both at acid and alkaline pH [in the latter case as aluminate, $Al(OH)_4^-$]. The hydroxide then matures, i.e., forms various denser crystal structures (gibbsite, bayerite), and gradually becomes less soluble. This maturation occurs very slowly and, under certain conditions, reaches equilibrium only after years (*1*).

In its complexes Al^{3+} is generally bound to oxygen, but nitrogen binding also occurs. The ligand exchange is slow partly due to a fairly marked covalent contribution

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to the Al-O and Al-N bonds. Slowness of the ligand exchange is also due to other things, e.g., that the exchange generally occurs by a dissociative mechanism (*2*) (see note 1, Appendix), and the complexes are often chelates. The slowness explains why the toxicity and other effects of Al^{3+} are easily underestimated in short-term experiments. The effects can neither be intensified or accelerated by increasing the Al^{3+} concentration, since this results in the formation of polynuclear complexes.

Al^{3+} has a strong affinity to phosphate, both inorganic free phosphate ions and various organic phosphate compounds. Moreover, Al phosphate is not an ordinary salt complex but partly a double oxide analogous to 2 SiO_2. Thus, crystalline Al phosphate and its hydrates have many properties similar to quartz and silicic acid.

Important properties of those ions that are chemically and biologically most closely related to Al^{3+} appear in Figure 1 and Table 1. The greatest similarity is, of course, shown by the ions immediately following Al in the same group of the periodic table. Two subgroups of higher analogs then exist, in one gallium and indium, and in the other scandium and yttrium (thallium, the lanthanides and the actinides also belong to the groups but chemically differ much more from Al). In the ionic state Sc^{3+} and Y^{3+} show the greatest formal similarity to Al^{3+}, all having electron clouds with inert gas configurations. Ga^{3+} and In^{3+} have, however, completely filled *d* orbitals, which in most cases afford the same qualities as the inert gas configuration. As shown in Table 1, all four of these ions have a lower charge/radius ratio than Al^{3+} and therefore their polarizing capacity is lower than that of Al^{3+}. The greatest similarity to Al^{3+} is shown by Sc^{3+} and Ga^{3+}. All three are mainly amphoteric, but Sc^{3+} is slightly more alkaline and Ga^{3+} slightly more acid than Al^{3+}. The solubilities of the hydroxides are roughly the same.

Boron is the first element in the same group of the periodic table as Al. However, boron only formally resembles Al and in its chemical properties is much more like the second element in the following group, silicon. In the same way, beryllium, the first element of the preceding group, is in many ways similar to Al. It is of special interest to observe that Be^{2+} has the same high charge/radius ratio as Al^{3+} (see Table 1), and the same electron negativity and solubility of the hydroxide. However, the lower charge and the normally lower coordination number of Be^{2+} are differences that should be reflected in the metabolism.

Among other "inert gas" ions, the titanium ion Ti^{4+} and the zirconium ion Zr^{4+} also resemble Al^{3+}, as does the rarer hafnium ion. The current literature on the biochemistry and metabolism of Ti^{4+} is more limited than the corresponding literature concerning Zr^{4+}. Therefore, the latter ion will primarily be discussed. Zr^{4+} has roughly the same high charge/radius ratio as Al^{3+} and Be^{2+}, but Zr^{4+} is somewhat more acidic than Al^{3+}, while Be^{2+} is somewhat more alkaline. In other respects as well (e.g., charge, radius, and coordination numbers) Al^{3+} is intermediately positioned between Be^{2+} and Zr^{4+}.

In this comparison Cr^{3+} and Fe^{3+} are also included, as they show many similarities to Al^{3+} in their inorganic chemistry and, in addition, their metabolisms have been studied in great detail. Important properties, differing from those of the other previously discussed ions, are the possible redox reactions and the incompletely filled *d* orbitals. The latter result in so-called ligand field effects that stabilize the binding of many different ligands, but which also increase the steric demands on these. If Cr^{3+} and Fe^{3+} are bound "nonspecifically" to a ligand, one can expect that Al^{3+} will also be bound to the same ligand but that the resulting complex is weaker. The same behavior should also apply to the other "inert gas" ions and the d^{10}-ions in question. However, the reverse does not necessarily apply; i.e., a ligand that binds Al^{3+} does not necessarily bind Cr^{3+} and Fe^{3+}. An example of these relations is the binding of Al^{3+}, Ga^{3+}, In^{3+}, Sc^{3+}, and Y^{3+} to transferrin (as well as Cr^{3+}, Co^{3+}, and Mn^{3+}, for reference see each respective section below). Another example is the inhibition of ferroxidase (ceruloplasmin) by Al^{3+}, Ga^{3+}, In^{3+}, Sc^{3+}, Y^{3+}, and Zr^{4+}, while at the same time ferroxidase is not inhibited by the transition element ions Cr^{3+} and Rh^{3+} (*4*). This "rule," however, should be less reliable in cases where Fe^{3+} (as actually in the examples above) participates in "specific" interactions with enzymes or biological transport systems.

Occurrence. On the average, Al^{3+} constitutes just over 8% (*w/w*) of the earth's crust, and the ion is a major component of a large number of minerals, e.g., mica, feldspar, and clays. At neutral pH, Al minerals are extremely insoluble, and the concentration of dissolved Al^{3+} is therefore low in both surface and subsoil water. However, solubility increases at lower pH levels. The current acidification caused by rain-borne nitric and sulfuric acid and the use of acidifying fertilizers increases the concentration of soluble Al^{3+} in soil and in lakes and rivers.

Table 1. Chemical and physical properties of Al^{3+} and similar ions compiled from various standard tables.

Cation	Al^{3+}	Ga^{3+}	In^{3+}	Sc^{3+}	Y^{3+}	Be^{2+}	Zr^{4+}	Cr^{3+}	Fe^{3+}
Ionic radius, Å	0.51	0.62	0.79	0.73	0.89	0.35	0.72	0.62	0.64 (0.55)
Charge density *z/r*	5.66	4.84	3.80	4.11	3.37	5.71	5.56	4.84	4.69 (5.45)
Coordination number	(4),6	6	6	6	6–8	(3),4	6–8	6	6
Electron configuration[a]	IG	$3d^{10}$	$4d^{10}$	IG	IG	IG	IG	$3d^3$	$3d^5$
Mean occurrence per ton of earth's crust	80 kg	10 g	< 5 g	5 g	≤ 5 g	10 g	200 g	100 g	47 kg
Suitable radionuclide	—	^{67}Ga	^{111}In	^{46}Sc	^{91}Y	^{7}Be	^{88}Zr	^{51}Cr	^{59}Fe
Half-life of this nuclide, days	—	3.3	2.8	84	59	53	85	28	45

[a] IG, inert-gas like configuration.

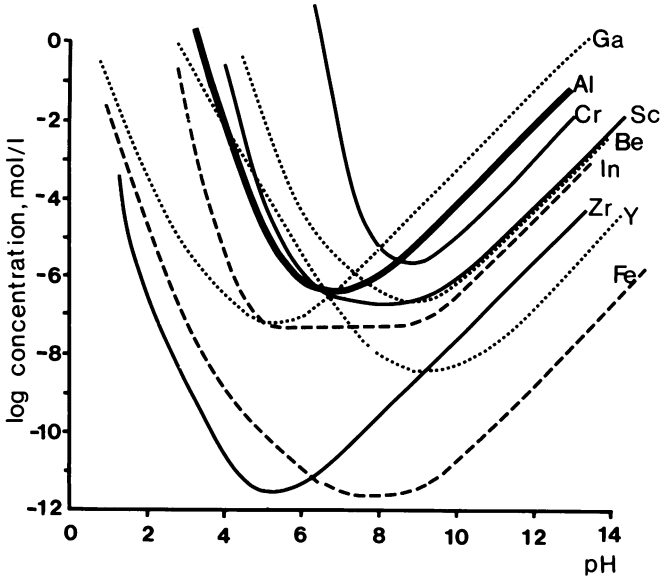

FIGURE 1. Maximal solubility of Al^{3+} and similar ions in water at various pH according to literature data (*3*).

Coexisting calcium minerals have a buffering effect that temporarily protects the Al compounds whereas the calcium compounds themselves are dissolved and leached. Differences in soil and rock composition cause variations in the concentration of dissolved Al^{3+} between 10 and 1000 μg/L in the lakes and rivers of Sweden, and many places also show very distinct seasonal variations in their recorded concentrations. Most probably the conditions are similar in many other industrialized countries. However, in sea water the concentration of dissolved Al^{3+} is low (< 1 μg/L), possibly due to the siliceous remains of diatoms that bind Al^{3+} (*5,6*).

Several reports concerning acidified lakes have shown (*7–9*) that it is not primarily the low pH but the subsequent increase of dissolved Al^{3+} in the water that causes gill damage and death among young fish. There are even indications that elevated levels of dissolved Al^{3+} in lakes have caused a serious and sometimes lethal intoxication in birds living in the immediate surroundings (*10*).

Al^{3+} is also toxic for most plant species, and dissolved Al^{3+} in the soil has been regarded as one of the foremost growth limiting factors in many parts of the world (*11*). Several mechanisms can help bring about this; the most important is, probably, the ready ability of Al^{3+} to bind and to prevent root uptake of inorganic phosphates. Al^{3+} also seems specifically to inhibit root growth (*12–14*). Ga^{3+} and In^{3+} have similar effects (*13*). However, many species have developed an ability to tolerate comparatively high concentrations of dissolved Al^{3+} (see note 2), and even within the same species this ability can vary considerably. Plant cultures that for many generations have been grown in Al^{3+}-rich environments have, by natural selection, developed special Al^{3+}-resistant varieties (*11*).

Al^{3+} is present in small quantities in most food (*15–17*), and both soluble and insoluble Al compounds are permitted as food additives in many countries. Small amounts of Al^{3+} are also released from Al cooking utensils and are dissolved in the food, particularly when the food products are acidic. Furthermore, Al^{3+} is commonly used in water purification and no maximal acceptable level for Al^{3+} in drinking water has been established in Sweden (concentrations vary between 10 and 500 μg/L). The total consumption of Al^{3+} in a normal diet is believed to be between 1 and 20 mg/day (*15,17–19*). Much larger amounts (1 g or more per day) are consumed by those taking antacids in which Al hydroxide is one of the main ingredients, and in one case a consumption in excess of 50g/day for several years has been recorded (*20*).

A concentrated solution of Al oxychloride [a hydrolysis and polymerization product of Al chloride at slightly acid pH, $Al_{13}O_4(OH)_{24}(H_2O)_{12}^{7+}$] is a very commonly used antiperspirant. Al salts have also been used as antiseptics (e.g., Al acetate, Burow's solution).

Finally, Al compounds are used in a large number of technical processes, e.g., as catalyzers in chemical synthetic industries, in paper industries, for the dyeing of textiles, etc.

Metabolism of Al^{3+}

Body Content and Absorption. No reliable information is available regarding the normal body content of Al^{3+} in humans. Reported investigations have mainly had the purpose of comparing either various tissues with one another or comparing healthy and diseased tissues. Therefore, absolute standardization has often been inadequate. A weighing of the evidence judged reliable (*18,21–26*) implies that the total body content for healthy individuals is about 30 to 50 mg. About half of this is present in the skeleton and one quarter is in the lungs.

With the exception of the lungs, all Al^{3+} in the body probably originates from food intake. Intestinal absorption is normally minimal, but direct investigations have been impossible due to the lack of suitable radionuclides. Reports of absorption fractions of about 10% or more (*27–29*) are most likely wrong, since they would suggest a rapid Al^{3+} accumulation in the body. The most credible information has been obtained by determining urinary excretion rates and regarding that as a minimal amount of absorption. Available information from the literature concerning normal excretion of Al^{3+} by the urine is summarized in Table 2. Since contamination gives higher values, the lower values shown in the table are probably more correct. Therefore, normal urinary excretion can be assumed to be 20 to 50 μg/day. If this is also accepted as a measure of the intestinal absorption and the daily intake is supposed to be 20 mg, then the normal uptake fraction would be about 0.1 to 0.3%.

Investigations performed have often concerned intestinal absorption after administration of large quantities of Al hydroxide and other Al compounds used as antacids. Daily administration of 2.2 g Al^{3+} as hydroxide increased the daily urinary excretion of Al^{3+} from 16 to about 300 μg (*34*). If one accepts the higher value as a measure of intestinal uptake, then this gives an absorption of about 0.01% at this elevated level of supply (*39,41*). As with

Table 2. Normal excretion of Al^{3+} in urine from adults, according to the literature.

Excretion, μg/day	Concentration, μ/L	Year	Reference
	46–110	1940	(*30*)
<30, median 17		1976	(*31*)
15–60[a]		1976	(*32*)
20–150		1977	(*33*)
16		1977	(*34*)
27–93		1978	(*35*)
20–80		1979	(*29*)
	6.4±4.5	1982	(*36*)
50–250		1983	(*37*)
	<15, median 4	1983	(*38*)
24–58		1983	(*39*)
	4.6±3.5	1984	(*40*)

[a] Occasionally up to 200 μg/day.

many other metal ions, the absorption of Al^{3+} probably increases when suitable complexing substances are also present in the intestine. Administration of the previously mentioned 2.2 g Al^{3+} as glycinate doubled the amount excreted in the urine in comparison with the hydroxide (*34*). Presence of phosphate prevented absorption, just as Al^{3+} hydroxide prevents phosphate uptake (*42*). It is postulated by one group (*43,44*), that the parathyroid hormone promotes Al^{3+} absorption, but these results have been called into question and are yet to be confirmed by others (*45–47*).

Occurrence in Blood Plasma. In plasma Al^{3+} is bound to transferrin, presumably to the same site as Fe^{3+} (*48–51*) but the extent to which it is normally bound is not known. Some current reports that about 50% of Al^{3+} is ultrafiltrable arise partly from experiments where the transferrin binding capacity has been exceeded by the addition of too much Al^{3+} (*52,53*). Some investigations carried out on patients with moderately increased serum concentrations (1–5 μmole/L) due to hemodialysis indicate that, at this concentration, only 10 to 30% of Al^{3+} is ultrafiltrable (*45,54,55*) and that the protein binding increases in relative terms as the concentration decreases (*55,56*). How the ultrafiltrable fraction is bound has not been investigated.

Reports in the literature concerning the normal plasma or serum concentrations of Al^{3+} vary considerably. The most credible values are presumably the lowest, 1 to 5 μg/L. To illustrate how undeveloped the research in this field has actually been, a compilation of the latest information is given in Table 3. It is notable that the lowest reported values during the last decade have decreased almost by a factor of 100. These variations probably reflect the difficulties that are connected with obtaining and handling the specimens more than with the actual determinations (*57*).

Tissue Distribution and Occurrence. Following the course of Al^{3+} in the body is difficult due to the lack of radionuclides. Experiments have, therefore, been conducted with stable ^{27}Al and have primarily had the purpose of elucidating problems regarding hemodialysis with Al contamined media. The plasma concentration of Al^{3+} has, consequently, often been much higher than in normal conditions. In such experiments with dogs, about half the Al^{3+} was eliminated from the plasma within 30 min, and one-third was excreted via the urine within 2 hr (*52*). Urinary excretion then declined rapidly, and it was therefore concluded that a large proportion of Al^{3+} had been bound to body tissues or excreted in other ways. However, Al^{3+} was not excreted in the bile, but it was believed possible that it was secreted via the mucous membranes or secretions of the alimentary tract (*52*). Rapid elimination of Al^{3+} from the plasma and subsequent binding to body tissues has also been illustrated by continuous intravenous infusion of Al^{3+} (dialysis) for a 1-hr period, which resulted in an increased level of Al^{3+} from the normal level of about 5 μg/L to a new one of just over 100 μg/L (approx. 4 μmole/L). This level then remained constant despite continued infusion during a 4-hr period. As soon as the dialysis was discontinued the level dropped again. A level of 4 μmole/L was equivalent to only about 10% of the normally available binding capacity of transferrin. Therefore, this experiment may fairly well reflect the normal plasma elimination process.

The normal occurrence of Al^{3+} in the body tissues has been studied in very few investigations, and in cases when the same organs have been examined, the results often vary considerably (*15*). It is therefore difficult to draw any unequivocal conclusions. The highest concentration is found in the lungs, probably around 20 mg/kg wet weight (*21–23,30,46,102*). The high Al concentration can, of course, be due to accumulation of insoluble Al compounds entering via the airways. Most other soft tissue organs have concentrations, according to the most credible reports, of about 0.3 to 0.8 mg/kg wet weight (*21,22,30*), i.e., 100 to 300 times higher than the probable concentration in blood plasma. Higher concentrations have been found in the skin (*21,46*), lower alimentary tract (*21*), lymph nodes (*22*), adrenals (*21,28,103,104*), and the parathyroids (*105*).

Many reports on the normal concentration of Al^{3+} in the brain have been published (Table 4). Some discrepancies are probably due to differences in the methods used. Otherwise, relatively good agreement exists establishing a normal level to between 0.25 and 0.75 mg/kg wet weight. The gray matter has been indicated to have about twice the concentration found in white matter (*113,122,123*). The concentration is reported to be higher in the small vessels and the meninges than in the surrounding brain tissue (*108,112*). Several authors have reported definite variations between different parts of the brain, but no systematic investigation has apparently been performed.

For bone tissue, the reported normal levels are more varied (*124*). Also here, the lowest recorded values are probably the more correct, 5 to 10 mg/kg, which is also in agreement with a later report (*24*) and means that bone tissue has the next highest concentration after the lungs.

Uremic patients, not receiving dialysis, show markedly increased Al^{3+} concentrations in the serum, bone tissue, liver, and spleen and a slightly increased concentration in the brain and skeletal muscles (*23,80,113*). Bone tissue, the liver, and the spleen can, then, presumably be the

Table 3. Concentration of Al^{3+} in plasma or serum from healthy individuals according to the available literature.

Concn. (mean or median value), μg/L	Range, μg/L	No. of individuals	Published year	Reference
240	—	pool	1940	(*30*)
172	±80	536	1960	(*58*)
—	400–450	266	1964	(*59*)
—	170–360	—	1966	(*60*)
240	—	5	1970	(*61*)
340	±190	21	1971	(*62*)
110	<60–790	105	1971	(*63*)
72	±70	10	1972	(*27*)
—	18–36	20	1973	(*64*)
37	10–90	29	1974	(*65*)
14	4–34.5	40	1976	(*31*)
7	±4	32	1977	(*34*)
—	4–15	9	1978	(*66*)
210	±20	—	1978	(*67*)
24.3	10–45	59	1978	(*68*)
25	10–50	10	1978	(*69*)
22	±9	10	1978	(*70*)
19	±6	11	1978	(*71*)
28	±9	23	1978	(*72*)
40.2	±7.2	10	1978	(*53*)
1.6[a]	0–50	20	1978	(*73*)
23	±7.3	20	1979	(*74*)
15	±12.6	44	1979	(*75*)
—	300–580	93	1979	(*76*)
140	±60	10	1979	(*77*)
50	—	1	1979	(*78*)
3	1–6	10	1979	*b*
49	±11	7	1979	(*79*)
6.2	1–15	31	1979	(*80*)
—	150–200	—	1979	(*81*)
2	0–6	20	1980	(*82*)
3.72	±1.20	8	1980	(*83*)
42	±16	20	1980	(*84*)
14.15	±12.2	44	1980	(*85*)
13.9	±6.2	20	1980	(*86*)
5.9	±2.3	8	1980	(*87*)
<4	<2.5–7	37	1981	(*88*)
7.7	2–15	45	1981	(*89*)
9.8	4.1–20	19	1981	(*90*)
14	3–39	54	1982	(*91*)
6.5	0–14.4	6	1982	(*92*)
10.8	±8.1	21	1982	(*93*)
14.4	9–39	36	1982	(*94*)
7.3	2–15	46	1982	(*95*)
35.0	±3.7	7	1982	(*96*)
8.4	±2.6	10	1982	(*36*)
6.5	2–14	28	1982	(*97*)
15.3	5–25	15	1983	(*37*)
2.7	±0.6	4	1983	(*57*)
6.1	1–12	50	1983	(*98*)
4	<2–11	8	1983	(*39*)
14.1	±12.2	44	1984	(*99*)
9.2	±2.2	50	1984	(*100*)
6.6	±2.6	10	1984	(*101*)

[a] Probable printing error. Should possibly read 16.
[b] P.O. Ganrot, 1979, unpublished.

primary depots for a beginning Al accumulation in the body under conditions where there is inadequate elimination. With infusion of large amounts of Al^{3+} over a long period of time (hemodialysis), the Al concentration strongly increases. In relative terms the increase occurs mostly in the spleen, followed by the liver and least of all in the skeletal bones (*23,46*). Due to the heavy weight of the skeleton and its relatively high initial Al concentration, the absolute increase was, however, greatest in the bones—about 2 g.

Very few data are found in the literature on any age differences of Al^{3+} concentrations in plasma or body tis-

Table 4. Normal concentration of Al^{3+} in the brain, according to the literature.

Species	Reported concentration, mg/kg dry weight[a]	Probable concentration, mg/kg wet weight[b]	References
Cat	1.4 ± 0.7	0.35 ± 0.18	(*106–108*)
Rat	1.1	0.28	(*106*)
—		0.77 ± 0.19	(*44*)
—[c]		7.1	(*28*)
—		<0.5	(*109*)
—		0.2	(*113*)
—	1.1 ± 0.4	0.28 ± 0.1	(*108*)
Rabbit	0.8 – 1.6	0.2–0.4	(*110*)
—	1.1 ± 0.3	0.28 ± 0.08	(*108*)
—	0.7 – 1.5	0.18–0.38	(*111*)
Dog	1.5 ± 0.4	0.38 ± 0.1	(*108*)
Rhesus monkey	1.6 ± 0.4	0.4 ± 0.1	(*108*)
Human	2.5 ± 0.3	0.63 ± 0.08	(*112*)
—	1.9 ± 0.7	0.48 ± 0.18	(*108*)
—[d,e]	2.4 ± 1.3	0.4 ± 0.22	(*23, 46*)
—[d]	0.9 ± 0.2	0.23 ± 0.05	(*113*)
—		<0.6	(*114*)
—		0.41 (0.1–3)	(*115*)
—	1.4 (0.9–2.4)	0.35 (0.23–0.6)	(*116*)
—		0.86 ± 0.12	(*117*)
—		1.5 ± 0.9	(*118*)
—		0.23 (0.18–0.26)	(*21*)
—		0.5 ± 0.1	(*22*)
—	11.9 (6.1–17.8)	3 (1.5–4.5)	(*102*)
—[d]	9.5 (4–14)	2.4 (1–3.5)	(*119*)
—	17 ± 8.2	4.3 ± 2.05	(*120,121*)
—[c]	15.2 ± 1.0	3.8 ± 0.25	(*67*)

[a] In cases where the concentration is reported only as dry weight, the value has been divided by 4 to give the concentration as wet weight (*21*).
[b] Concentration per fat-free dry weight has, in the same way, been divided by 6.
[c] Authors report correspondingly high values in other organs.
[d] In gray substance.
[e] As fat-free weight.

sues. Increases with age have been reported for lung tissue (*46,103,125*), the liver (*103*), the kidneys (*103*), and the brain (*112,115,126*).

Intracellular Metabolism. Scarcely any investigations have been performed to reveal the mechanism for Al^{3+} uptake in cells. One investigation, utilizing the short-lived isotope ^{28}Al (half-life 2.3 min), gave an indication that some form of specific mechanism was present but conclusive evidence was lacking (*122*).

Intracellular localization has, on the other hand, been studied by many investigators (Table 5). The general opinion is that Al^{3+} is mainly bound to the cell nucleus, but one group has instead reported that Al^{3+} was localized in the lysosomes and that Al^{3+} was not detected in other structures (*109,133,136–138*). This group, utilizing an ion-probe technique, has also demonstrated that Al^{3+} exists together with equimolar amounts of phosphorus and has, therefore, assumed that Al^{3+} which is present in lysosomes is crystalline Al phosphate having a molar ratio 1:1. A possible reason for the varying opinions regarding intracellular localization of Al^{3+} can be differences in the methods used and their varying abilities to detect different Al compounds. Microprobe techniques require a local, relatively high fraction of the atoms, that are to be detected, and therefore detect Al^{3+} in crystalline phosphate easiest. In contrast, histochemical techniques depend upon accessibility to well-isolated Al ions for the chelating dye reagent to work. Histochemistry should, then, be more insensitive for crystalline Al^{3+} but more sensitive for Al^{3+} bound randomly in the chromatin (*142*). Other possible causes for the varied results could be differences in the experimental administration of Al^{3+} and in the handling of the specimens prior to analysis. No studies directly investigating the fate of intracellular Al^{3+}, its elimination from the cell or turnover in different cell compartments have been conducted. Some indirect information from similar ions is discussed below.

Biochemistry of Al^{3+} and Its Effects on Cell Metabolism

Effect on Enzymes and Related Substances. Only a few conclusive examples of effects on enzymes have been published and many of these effects have been demonstrated with Al^{3+} concentrations probably alien to the organisms. Very often, the experimental conditions have been such that the ion must be assumed to have been colloidal. A detailed review of known enzyme effects has been given elsewhere (*143*).

Table 5. Dominating intracellular localization of Al^{3+} according to the literature.

Species	Cell type	Localization	Technique	Reference
Onion	Root	Nucleus	Histochemistry and chemical analysis	(*12*)
—	Root	Nucleus	Histochemistry	(*128*)
Pea	Root	Nucleus and cell wall	Histochemistry	(*129*)
—	Root	Epidermis	Electron probe	(*129*)
Bean and cotton	Root	Nucleus and cell wall	Electron probe	(*130*)
Frog	Liver, skin	Nucleus	Ion probe	(*131*)
Rabbit	Thyroid	Nucleus	Ion probe	(*131*)
Rat	Brain	Lysosomes	Ion probe	(*109*)
—	Renal tubulus	Lysosomes	Ion probe	(*133*)
Cat	Brain	Nucleus	Histochemistry	(*128*)
Human	Histiocytes	Lysosomes	Electron probe	(*132*)
—	Lymphocyte	Nucleus	Histochemistry	(*128*)
—	Brain	Nucleus	Chemical analysis	(*106*)
—	Brain	Nucleus	Electron probe	(*134*)
—	Brain	Nucleus	Histochemistry	(*135*)
—	Liver	Lysosomes	Ion probe	(*136*)
—	Parathyroid	Lysosomes	Ion probe	(*137*)
—	Brain	Lysosomes	Ion probe	(*138*)
—	Brain	Nucleus	Electron probe	(*139*)
—	Spleen, liver	Lysosomes	Laser probe	(*140*)
—	Liver	Lysosomes	Laser probe	(*141*)

The most well-documented effect is probably the inhibition of hexokinase (*143–148*), which is caused by the tendency of ATP to form stronger complexes with Al^{3+} than with Mg^{2+} and by the fact that the Al^{3+}–ATP complex acts as a strong competitive inhibitor with respect to Mg^{2+}–ATP. The interaction between Al^{3+} and ATP (*149*) may imply that Al^{3+} can affect many other enzyme reactions where ATP is a substrate, e.g., $Na^+ + K^+$-ATPase (*150*). Among other enzymes that are inhibited by Al^{3+} are adenylate cyclase (*151,152*), 3′,5′-cyclic nucleotide phosphodiesterase (*153*), alkaline phosphatase (*154,155*), acetylcholinesterase (*156,157*), serum cholinesterase (*158*), catechol-O-methyltransferase (*159*), and ferroxidase (*4*). Both skeletal acid and alkaline phosphatase are activated by Al^{3+} at concentrations between 10^{-11} and 10^{-6} mole/L but are inhibited at higher concentrations (*160*).

The inhibition of phosphodiesterase is probably the result of a high-affinity interaction between Al^{3+} and calmodulin, the normal activator of the enzyme. Al^{3+} has been shown to bind stoichiometrically and cooperatively to calmodulin and thereby to induce a major structural change (*153,161*). So far the effect has only been studied *in vitro* but in view of calmodulin's role as a regulator of many cell enzymes the effect on calmodulin could in the future prove to be among the most important ones.

One group has reported that Al^{3+} inhibits the uptake of some neurotransmitters in isolated synaptosomes *in vitro*; namely, γ-aminobutyrate, L-glutamate (*162*), choline (*163*), noradrenalin, and serotonin (*164*). The effect was strongest on the choline uptake (for 50% inhibition, 24 μmole/L was needed) and weaker for the others (200–300 μmole/L was needed for 50% inhibition). Al^{3+} has also been reported to form complexes with (Leu^5)-enkephalin (*165*).

Among enzymes reported to be activated by Al^{3+} are ALA-dehydratase (*166*), phosphoglucomutase (*167*), succinic dehydrogenase (*168,169*), and trypsin (*170*). These effects are, however, not fully investigated or documented.

Effect on DNA and Cell Division. Much points towards the chromatin and DNA being the cell structures most vulnerable to Al^{3+}. The ion has a very high affinity to DNA, as well as to RNA and many mononucleotides; but it has apparently been difficult to find satisfactory methods to study the effects on DNA and few investigations have been published.

Exactly how Al^{3+} is bound to DNA is still uncertain. Probably, it is bound only to the phosphate groups (*171–173*). The amount of Al^{3+} bound is extremely variable. Possibly a complex with an Al/DNA-P ratio about 1:3 is first formed. However, excesses of Al^{3+} can increase the ratio to greater than 2:1, most likely due to binding of OH-ions as well (*129*). The ratio can then, possibly, increase unlimited by the complex acting as a crystallization nucleus for Al hydroxide.

Histones, and, to some extent Ca^{2+} and Mg^{2+}, are bound *in vivo* to the DNA phosphate groups. In an *in vitro* study that gave an Al/DNA-P ratio of about 1:3 with histone-free DNA, added histones blocked about 80% of the Al^{3+} binding (*129*). If an excess of Al^{3+} can also reduce the histone binding to DNA is unknown.

In the chromatin, histones and divalent cations reduce the negative charge of DNA, thereby decreasing the repulsive forces that otherwise exist between the strands of DNA. The stability of the double-strand structure is usually studied by measuring the "melting point" of DNA, i.e., the temperature at which the hydrogen bonds between the bases are split. The melting point can be demonstrated spectrophotometrically. Mg^{2+} raises the melting point of double-strand histone-free DNA. In one investigation, using pea DNA, no rise was detected with

Al^{3+}, but instead a dose-dependent reduction of the "hyperchromicity" which marks the melting, was observed. It was concluded that Al^{3+} had probably been bound only to part of the DNA and this part was then so strongly stabilized, that it could not melt under the circumstances used. The remaining DNA was unaffected (*171*).

With other experimental conditions (e.g., calf thymus DNA), another group reported that a low concentration of Al^{3+} (Al/DNA-P = 0.6) stabilized the double-strand structure and raised the melting point, while higher Al^{3+} concentrations (Al/DNA-P > 1), instead destabilized the structure (*71*). However, in later studies by the group (*173,174*), where the pH effects were particularly studied, the findings have compared well with the results of the first group (*171*). Thus, at physiological pH and with Al/DNA-P ratios between 0.6 and 0.7, a small portion of DNA melted at the same temperature as Al^{3+}-free DNA (about 60°C) while another portion melted only at 90 to 100°C. At low pH ($\leq$ 6) a destabilization of the double-strand structure of DNA was observed instead.

All the cited investigations were performed, for technical reasons, on very dilute DNA solutions, 40 to 60 μmole/L, expressed as mononucleotides. In the cell nucleus, DNA is present in at least a 1000 times greater concentration, 20 to 200 mmole/L, and even higher in heterochromatin. At the same time, the Al/DNA-P ratios used were about 10 times that of the highest *in vivo* values recorded in severe Al^{3+} intoxication (*106*). Therefore, if the purpose of the investigations was to reflect conditions present *in vivo* in the cell nucleus, the DNA concentration should have been 1000 times and the Al^{3+} concentration 100 times greater. Thus, the experimental conditions have, undoubtedly, greatly reduced the complexation tendency between DNA and Al^{3+} and also reduced the polymerization tendency of the ion. If the above-mentioned, higher concentrations had been used, this would immediately have led to an amorphous precipitation of Al hydroxide and Al DNAate that would then have been impossible to study. However, it is possible that this more coarse precipitating effect of Al^{3+} on DNA is, biologically, more important than any "over-stabilization" of the double-strand structure.

In studies of RNA synthesis *in vitro*, it has also been necessary to work with very low concentrations of DNA, 10^3 to 10^4 times lower than in native cell nuclei, and with high Al/DNA-P ratios (*171,175,176*). If Al^{3+} was supplied *in vivo* (pea roots incubated one day with $AlCl_3$, 1 mmole/L at pH 4.5), raising the Al/DNA-P ratio to 0.086, then RNA synthesis was half of that without Al^{3+} and the effect was distinctly dose-dependent. However, if Al^{3+} was added *in vitro* to isolated chromatin and was incubated 1 hr prior to the experiment, a lower effect on RNA synthesis was noted with an identical Al/DNA-P ratio. The effect of Al^{3+} on the fidelity of DNA synthesis *in vitro* has also been studied with low concentrations of DNA [low molecular weight poly(dA-dT)] and with such high Al^{3+} concentrations (10 mmole/L) that almost all Al^{3+} must have existed as colloid. No reduction of the fidelity was observed (*177*).

In vitro Al^{3+} (0.5 mmole/L) inhibits RNA synthesis by isolated rat brain cell nuclei (*178*) and also inhibits "puffing" of polytene chromosomes from the saliva gland of fly larva (*179*). Furthermore, neuroblastoma cells, cultured in an Al^{3+} environment, showed a slightly reduced RNA synthesis, but it is uncertain if this was a direct effect (*180*). Al^{3+} has also been reported to inhibit the binding of corticosterone–receptor complex to DNA (*181*). The experiments were performed *in vitro* with rabbit neurons but the Al^{3+} was supplied *in vivo* before the experiments.

The effect of Al^{3+} on the root filament growth of plants has been regarded as a measure of impaired DNA function (*11,13*). Al^{3+} has also been reported to impair cell division in plants (*12,13*). The more precise mechanisms governing these effects, however, have not been investigated. A cytological examination of the root meristem of the onion showed that Al^{3+} and several other ions, e.g., Fe^{3+}, Cr^{3+}, Be^{2+}, Y^{3+}, and Zr^{4+}, made the chromosomes "sticky" and resulted in anaphase bridges. Signs of mitotic spindle disturbances were also present, often with typical colchicine mitosis (*182*). Under certain experimental conditions, Al^{3+} has even been reported to cause chromosomal breakage and other chromosomal mutations in plants, both during mitosis and meiosis (*183,184*). One investigation reported Al^{3+} not to be mutagenic in tests with *B. subtilis* (*185*), but the ion must have existed as a colloid in these experiments.

Al^{3+} and its compounds are generally regarded not to be carcinogenic, but still there are reports that subcutaneous implantation of Al foil in rats (*186*) or subcutaneous injections of Al dextran in mice (*187*) have produced high frequencies of sarcoma.

Effect on Microtubules and Filaments. Experimental intoxication with Al^{3+} can cause neurofibrillary degeneration (NFD) in cats (*107,108*) and rabbits (*109,110,189–193,205*), and in rabbits it has even been produced by metallic Al powder (*194*). In other species, e.g., humans and rats, Al^{3+} seems not easily to produce NFD *in vivo* but it has been induced in cell cultures with fetal human cerebral cortex cells (*195*) and with neuroblastoma cells from mice (*180*). NFD is a fibrillary deposit near the neuronal cell nucleus consisting of packed 10 nm protein filaments. The same type of NFD can also be induced by colchicine and similar mitotic spindle inhibitors (*196–198*) and by maytansinoids (*199*).

Al^{3+}-induced NFD has been observed, mostly, in very large neurons, e.g., in the anterior horn cells of the spinal cord, the giant pyramidal cells of the cerebral cortex, and the Purkinje cells in the cerebellum (*107,110,192,200*). Parallel to the increase of fibrillary deposits there is also seen a strong decrease (about 20 times) in the number of microtubules in the affected neurons (*188*), as well as a dendritic dying back (*191*). The volume and protein content of the neuronal perikarya are also reported to increase (*189,201*).

Analysis of the structure of accumulated fibrils induced by colchicine and Al^{3+} showed that the proteins that constitute the normal neurofilaments dominate (*189,202–204*). Why they accumulate in this way is not fully understood; possibly it is due to a primary microtubular dys-

function. Microtubules are believed to be in a dynamic state of continuous assembly and disassembly and this process is probably essential for their function. Colchicine prevents polymerization of tubulin to microtubules and also inhibits many intracellular transport functions, in which microtubules take part, e.g., cell division, axonal transport, and release of secretion granules from various cells. Possibly, the inhibition by Al^{3+} or colchicine may also prevent the transport to peripheral parts of the neurons of the newly formed neurofilament proteins, which then, instead, may aggregate close to the site of production. This assumption is supported by the fact that neurofibrillary deposits are detected in the proximal end of the axon only one day after administration of Al^{3+}, while it takes several weeks before NFD is seen near the nucleus (*205*). During this latency period the neurons show a progressive loss of excitability (*111*). Therefore, cell damage is conceivably not caused by the presence of fibrillary deposits, *per se*, but by the loss of the normal functions of neurotubules and neurofilaments.

Al^{3+} has been reported to inhibit polymerization of tubulin to microtubules both *in vivo* in a protozoa and *in vitro* with neuronal tubulin (*206*), but the Al^{3+} concentrations used were high (0.1–10 mmole/L). The physiological relevance might therefore be doubtful, although the results are in accordance with the above mentioned reduction of microtubules in neurons with Al^{3+}-induced NFD. Al^{3+} and several other similar ions are also reported to have an effect on cell division in plants, which resembles the effect exerted by colchicine (*182*). The likeness of the effects exerted by Al^{3+} and colchicine are further illustrated by two investigations where NFD was induced by Al^{3+} (*180*) and colchicine (*207*). In both cases the ^{14}C-leucine incorporation in cell proteins increased but the concentration of RNA in the neurons decreased. For colchicine, this paradox was attributed to the protein synthesis being reduced. However, simultaneously the transport of this protein from the cell center was even more drastically reduced.

Effect on Lipid Membranes. Al^{3+} is reported to decrease the fluidity of lipid membranes in *Thermoplasma acidophilum* (both isolated membranes and whole organisms), probably by replacing Ca^{2+} or Mg^{2+} as counterions of the phospholipids (*208*). The effect was demonstrated within the pH range 2 to 5. The choice of organism and pH was necessary to avoid the problems of insolubility and slow ligand exchange of Al^{3+} in neutral solutions. The lower pH level corresponded to the normal environment for the organism. At this pH, the effect of Al^{3+} was insignificant, probably due to phospholipids being less ionized. The effect increased continuously, with increasing pH up to pH 5, where Al^{3+} became insoluble. A level of 10 μmole Al^{3+}/L at pH 4 lowered the membrane fluidity equivalent to a 3°C decrease in temperature. The results suggest that the effect could be even greater at a more neutral pH, if Al^{3+} had adequate time to bind in sufficient quantities to the membrane.

Al^{3+} is also reported to inhibit capping in mouse lymphocytes. The effect was demonstrated at pH 6.0 to 6.2 with fluorescein-labeled anti-mouse IgG. The effect could be demonstrated at 10 μmole/L, and 50% inhibition was obtained with 70 μmole/L. Be^{2+} had an even greater effect (*209*).

Metabolism and Effects of Similar Ions

Gallium. Of ions similar to Al^{3+}, Ga^{3+} is one that has been most thoroughly investigated regarding its metabolism. This is due to the existence of a radionuclide, ^{67}Ga, that has been often used in nuclear medicine for detecting inflammatory lesions and many types of tumors. ^{67}Ga has a half-life of only 78 hr and therefore is not suitable for extended long-term studies. For scanning procedures, $^{67}Ga^{3+}$ is generally given intravenously as a citrate solution and this form of administration has also mostly been used in studies performed with animals.

Ga^{3+} is absorbed only in very small amounts when administered orally (*210,211*). However, in mouse experiments the uptake of Ga^{3+} and In^{3+} by the duodenal mucosa was comparable to that of Fe^{3+}, but in contrast to Fe^{3+}, they were not transferred to the blood but were probably returned to the intestinal lumen as the mucosal cells died and were sloughed (*212*).

During the first few days after IV infusion of Ga^{3+} (e.g., for Ga scanning), roughly 25% is excreted in the urine and 10% in the feces. If an amount is administered, equivalent to 10 times the transferrin binding capacity, about 65% is excreted in the urine during the first 24 hours (*213*). About half the intravenously infused Ga^{3+} (trace dose in rats) binds to various body tissues (*214*), but with infusion of larger quantities the retention is significantly less (*215*). In Ga scanning, an accumulation of Ga^{3+} in the intestine is observed, usually localized in the intestinal wall rather than in the lumen (*216*). Ga^{3+} found in the intestinal lumen is believed to originate from the intestinal mucosa (*210,217*). Bile duct ligation did not reduce the excretion (*210*).

In plasma, presumably as much as 98 to 99% of Ga^{3+} is bound to transferrin (*218–220*). The binding is probably analogous to the Fe^{3+} binding (*49*), but the high binding constant is not as extreme (*219*). Transferrin strongly increases the uptake of Ga^{3+} in various tumor cell lines (*221–228*). Therefore, the uptake is assumed to be very similar to the uptake of Fe^{3+}–transferrin, i.e., Ga^{3+}–transferrin is first bound to a transferrin receptor and then, for most cells, uptake occurs probably by endocytosis, whereby the whole Ga^{3+}–transferrin complex is internalized in the cell and the ion is confined in a lysosome (*224*). Transferrin seems to be necessary for the uptake of Ga^{3+} (*225*). In the presence of transferrin, the uptake is considered to be dependent mainly on the number of transferrin receptors per cell. Fe^{3+} competes with Ga^{3+}; excess Fe^{3+} completely blocks the uptake of Ga^{3+} (*226*).

The high affinity that various types of tumors have to Ga^{3+} is believed to be due to the many transferrin receptors present on tumor cells (*221,225,227*) (active cell growth is presumably dependent on a rich supply of Fe^{3+}—with few transferrin receptors the tumor cell growth is inhibited). Tumors emanating from organs that

are normally particularly well furnished with receptors, e.g., choriocarcinomas, have an especially high affinity to Ga^{3+} (*229*). Lymphoid cells have a high affinity to Ga^{3+} and, therefore, the main clinical application of Ga^{3+} is in detecting various types of lymphomas and leukemic infiltrates (*230*).

Ga^{3+} accumulates in inflammatory lesions due probably to its high affinity to macrophages and various leukocytes. Granulocytes are believed to take up most of the nuclide (*231,232*). According to one hypothesis (*233,234*), this uptake is due to the presence, in the granules, of lactoferrin to which Ga^{3+} is reported to have a higher affinity than to transferrin (*235*). According to others, however, Ga^{3+} is bound mostly to the granulocytic cell membrane and can be almost totally removed by trypsin (*236*). This conception is, however, based on *in vitro* studies in a system that contained little, if any, transferrin. This near absence of transferrin may have influenced the mode of binding.

The distribution of Ga^{3+} in various organs after intravenous infusion has been investigated post mortem in patients that received Ga^{3+} shortly before their death. An early study (*237*) concerned 13 individuals that died within 72 hr after infusion with $^{72}Ga^{3+}$. The highest concentration was found in the kidneys. Of the other tissues, the liver, spleen, lymph nodes, bone marrow, and bone showed the highest concentrations. High concentrations were also found in the adrenals and skin. Another investigation (*214*) concerning 25 individuals who died within the first 20 days after $^{67}Ga^{3+}$ infusion showed an identical distribution. Comparable investigations have also been done in healthy rats (*215,238*). A very similar distribution was obtained with high values for liver, spleen, lymph nodes, and the skeleton, and low values for muscle and brain tissues.

The distribution of Ga^{3+}, according to these reports, is in agreement with the results obtained by $^{67}Ga^{3+}$ imaging in healthy subjects. In addition, $^{67}Ga^{3+}$ scanning has revealed a very high uptake in lactating breasts (*239*) and in placenta (*240*). A high uptake is often seen also in parathyroid glands (*241*), lacrimal glands (*242*), and salivary glands. In children, a large uptake is observed in the thymus (*243*) and in epiphyseal plates in the skeleton (*243*).

A Ga^{3+} whole-body retention study in humans suggested the presence of two compartments, one small with a short biological half-life and another, that bound just over 80% of the dose, with a biological half-life of 20 to 30 days (*244*). However, no measurements were possible after 17 days due to the nuclide's short physical half-life. Therefore, there is a possibility that compartments can exist with much longer biological half-lives.

In cell studies the first days after administration, Ga^{3+} has been found mostly in the lysosomes (*245–247*), possibly as crystalline Ga phosphate (*248*). In liver cells, it has been reported to be incorporated also in ferritin (*249,250*) but the evidence is not conclusive, and differing opinions exist (*228,251*). However, *in vitro* ferritin easily binds Ga^{3+} (*252*).

Due to the fact that Ga^{3+} is toxic and accumulates in several tumors, Ga compounds have been tested for any possible antitumor activity. The experiments have been performed both in humans and in rats and have been considered promising but have also shown that administration of Ga^{3+} in amounts far in excess of the transferrin-binding capacity results in acute renal damage (*213,253–255*). Anemia was also regularly induced by the administration (*213*). Moreover, Ga^{3+} had a rather specific toxic effect on cellular immunity in guinea pigs, that aggravated tuberculosis and inhibited the tuberculin test (*256*). After administration of Ga^{3+} to rats equivalent to LD_{50} (about 50 mg/kg body weight IV or 120 mg/kg SC), the surviving animals showed various neurological symptoms (*257*).

As previously stated, oral administration of Ga^{3+} for comparatively short periods showed a very low toxicity. Long-term administration (18 months), however, seemed to shorten the life span of mice and to cause an increased tumor frequency (mostly lymphomas and leukemia). However, the effects were not statistically significant (*258*).

Indium. In radionuclides have been used for many diagnostic purposes in nuclear medicine (see note 3). This has stimulated an interest in the metabolism of In^{3+} and resulted in a fairly extensive literature, but its biochemistry is largely uninvestigated.

After oral administration, In^{3+} is poorly absorbed (*212*). However, as with Ga^{3+}, a certain uptake is reported in the intestinal mucosa (*212*). In plasma In^{3+} is bound to transferrin, probably to a large extent (95%) (*259*). The association constant is higher than for Ga^{3+}–transferrin and is reported to be of the same order of magnitude as for Fe^{3+}–transferrin (*219*).

The distribution of In^{3+}, after intravenous administration, is dependent on the amount given, on the chemical properties of the compound in question, and on the transferrin saturation level. Injected intravenously as colloidal hydroxide the In^{3+} is taken up by and damages the liver, spleen, bone marrow, thymus, and lymph nodes (*260*). Administered as soluble salts and complexes, about 30% is excreted in the urine within 24 hr (*260,261*). The remaining portion is taken up mainly by the connective tissues including the skeleton (*261–263*).

Likewise, the final elimination, after IV administration in the mouse, is reported to be dependent on the amount given and on the chemical form (*260*). About 80% of soluble salts administered in moderate amounts were excreted within 2 weeks, two-thirds via the urine and one-third via the feces. Increased dosage decreased the fraction excreted somewhat. Colloidal hydroxide, however, was excreted considerably slower and only about one-fifth was via the urine and four-fifths via the feces. After 1 month, about 60% had been eliminated, but excretion via the feces was still about 4% per week.

A large proportion of soluble In^{3+} and In^{3+}–transferrin is taken up by the bone marrow, and this has, according to many clinical investigations, a certain value in assessing erythropoiesis. These reports have then led to several detailed studies on In^{3+} uptake by reticulocytes (*261,264,265*), which have shown that In-transferrin is first bound to the reticulocyte transferrin receptors in

the same way and with roughly the same affinity as Fe–transferrin. If the cells are then transferred to plasma lacking In^{3+}, almost all the bound In^{3+} can, contrary to bound Fe^{3+}, be washed off the cells. The In^{3+}–transferrin complex, therefore, remains membrane-bound, and a very small fraction of it is transferred into the cells. After about one week, only 1 to 2% of the administered In^{3+} dose is detected in the circulating erythrocytes compared to 85 to 90% for Fe^{3+}. However, Fe^{3+} uptake is inhibited by the binding of In^{3+}-transferrin to the transferrin receptors (*265*). No indications exist that In^{3+}, taken up by the erythrocytes, is ever bound to heme.

The behavior of In-transferrin in the rat placenta has also been studied in some detail (*266,267*). The placenta has a high affinity to In-transferrin but, contrary to Fe^{3+}, hardly any In^{3+} is transferred to the fetus; instead, it is accumulated in the placenta. According to one investigation, in late gestation the placentas accumulated within 5 hr about 40% of an IV dose of $^{113m}In^{3+}$ (*266*). In experiments on pregnant hamsters, intravenous In^{3+} induced various fetal malformations, mostly skeletal deformities, demonstrating some transfer to the fetus. Doses in excess of 1 mg/kg resulted in fetal death. In^{3+} also appeared to be more toxic than Ga^{3+} (*268*). If transferrin was saturated with Fe^{3+}, however, the embryotoxic effects of In^{3+} were blocked (*269*).

The intracellular binding of In^{3+} has been studied in various experiments with rats. Due to the short half-life of the radionuclides used, the studies were of only a few days' duration. In liver cells, about 35% of the radioactivity was found in the lysosomes and somewhat less in the cell nuclei. Of the radioactivity present in the cell supernatant, 32% was precipitated by antiserum against ferritin (4% of the total radioactivity of the cells), indicating that In^{3+}, to some degree, was bound by ferritin (*261*). Localization to lysosomes and cell nuclei has also been confirmed by other investigations (*270,271*). In renal tubular cells, In^{3+} (as well as Al^{3+}) was detected in the lysosomes by electron microscopy with secondary ion mass spectroscopy (*133*). In contrast to Al^{3+}, a preponderant binding to the cell nucleus seems never to have been observed. However, this could be due to the fact that only short-term experiments have been performed. In^{3+} has, namely, a very high affinity to DNA. Due to its comparatively high atomic weight, In^{3+} has therefore been proposed as a selective "staining" agent for DNA in electron microscopy (*272*).

Several studies on the toxicity of In^{3+} have been accomplished (*260*). Long-term oral administration in mice (5 ppm in water) had no detectable effect on the health or life span of the animals (*258*), nor could any effects be detected in rats with a single parenteral dose of up to 67 μg In^{3+}/kg. However, a 10-fold increase in that dose gave weight reduction and resulted, biochemically, in a reduction of the blood activity of alkaline phosphatase (*273*). Other findings in conjunction with more severe In^{3+} poisoning, are reports of anemia (*275*), paresis and other neurological disturbances (*275,276*) and serious renal damage (*260*), i.e., the same general symptoms that occur with severe Ga^{3+} poisoning. However, the picture is dependent on the compound administered (*260*).

Scandium. Sc^{3+} is present only in small amounts in the environment and has no common technical application. It is absorbed poorly by the intestine (*277*). Radionuclides have been tested for use in nuclear medicine but do not seem to have any advantages over, for example, ^{67}Ga. The long half-life of ^{46}Sc (84 days) is, moreover, a disadvantage in scanning studies, as it gives a higher exposure to radioactivity, but it is instead good for long-term metabolic studies. This characteristic has been exploited by plant physiologists that have used ^{46}Sc as a substitute radionuclide for Al (*278*).

In plasma, Sc^{3+} is bound to transferrin, and the binding seems to be completely analogous to the binding of Fe^{3+}, only weaker (*279*). *In vitro*, Sc^{3+} also binds to ferritin but the only known investigation *in vivo* did not confirm this, except possibly for a very limited quantity (*280*).

Several investigations have been performed with mice and rats regarding organ distribution after intravenous administration of Sc^{3+} (*281–284*). The results compare well and show that the chemical form used for the administration is of great importance (*284*). If Sc^{3+} is given as chloride or a weak complex (citrate), most of it is taken up by the reticuloendothelial system; stronger complexes (nitrilotriacetic acid, NTA) are bound more to the skeleton, and very strong complexes (ethylenediaminetetraacetic acid, EDTA) are completely excreted in the urine. The distribution changes considerably with time. Initially, the concentration is high in the lungs and kidneys. After 3 to 24 hr it is highest in the liver and spleen (*283*), and after 72 hr highest in the skeleton, but still high in the reticuloendothelial system (*282*). After 11 months, the concentration is highest in the spleen and bone marrow and appears to have diminished in the liver and skeleton (*281*).

A less detailed investigation has also been performed on three patients, who died 5, 6, and 7 months after Sc radionuclide administration (*285*). Among the investigated organs the spleen showed by far the highest uptake; this was followed by the uptakes in the liver and skeleton.

In this above-mentioned human study (*285*) and in one of the studies with rats (*281*), the retention after intravenous administration of Sc^{3+} was also followed by whole-body counting. Elimination was, of course, greatest in the period immediately following the injection and was soon followed by a very slow phase. The biological half-life, calculated on this phase, was 1300 and 1557 days, respectively, for two of the patients and 485 to 1200 days for the rats, depending on whether the administration was done with or without a stable Sc-carrier. The pool with this slow elimination in humans was about 90% of the administered dose, and in rats about 60%. The calculation of the half-life was based on the simple assumption that the pool with the slow elimination had a uniform half-life. However, this was not confirmed by the experiments, which continued only 350 to 500 days and therefore followed the elimination of only the first 20% of the pool. The assumption also appears unlikely; much more

probable is that the remaining 80% has had a longer biological half-life, and part of it much longer.

The reported investigations should imply that Sc^{3+} is accumulated in the body, at least in some compartments. Already a half-life of 1500 days for a compartment means that it takes about 14 years to achieve 90% of the final equilibrium value if the supply is continuous and nothing prevents the retained amount from increasing. Moreover, one investigation (*286*) has suggested that in humans the concentration of Sc^{3+} increases in various organs during life (lungs, heart, liver, kidney, brain); another (*287*) has stated that Sc^{3+} may increase in the brain with age.

It seems that few investigations have been performed to demonstrate how Sc^{3+} is bound in the cells or by the extracellular structures. In plants, cell nuclei have been said to accumulate Sc^{3+} (*278*), and glycosaminoglycans bind Sc^{3+} so strongly that it has been possible to use this binding in a method to determine these substances (*288*).

Biochemically, Sc^{3+} has been reported to inhibit acetylcholinesterase (*157*) and adenylate cyclase (*289*) and to affect the polymerization of G-actin to F-actin (*290*) together with Y^{3+} and several lanthanide ions.

Yttrium. In aqueous solutions, Y^{3+} is by far the most alkaline of all the ions compared, and in this respect is more like the lanthanides than the other Al^{3+}-like ions. Y^{3+} is sparse in the natural environment and has limited technical applications, primarily in electronic components. Y is formed in uranium fission (^{90}Y is the daughter nuclide of ^{90}Sr), and this has led to investigations regarding its metabolism, but unfortunately, most often with methodologically weaker techniques.

In the gastrointestinal tract, uptake is low and Y radionuclide salts have therefore been used in resorption studies as a nonabsorbable reference (*291*). Despite its scarcity and low uptake, Y^{3+} is usually detected in the body. The highest concentration has been reported in the lymph nodes and lungs (*22*). The behavior of Y^{3+} in blood plasma has not been elucidated. The distribution of IV-administered Y^{3+} is very similar to the distribution of Sc^{3+}. With administration of only trace amounts, most is bound to the skeleton; if more is administered, a large proportion is bound in the liver, spleen, and bone marrow (*292–294*). The type of Y^{3+} compound used is also important for the distribution: Y-EDTA is excreted in the urine or bound in the skeleton, Y-NTA is bound in the skeleton or liver (*284,295*), and Y citrate or Y chloride is mostly bound in the liver. In the skeleton, most of the ion is bound in the epiphyseal plates (*296,297*) and the binding is believed to be very stable, as bound Y^{3+} was detected unchanged after 6 months (*297*). The intracellular distribution has shown a concentration to cell nuclei in several organs, while in other organs a more diffuse distribution has been noted (short-term study) (*298*).

Very few studies have been performed regarding the possible toxic effects of Y^{3+} or its biochemistry, but it is reported to inhibit succinic dehydrogenase, Ca^{2+}–ATPase (*299*) and acetylcholinesterase (*157*).

Beryllium. The general chemistry of Be^{2+} is considered to be very similar to that of Al^{3+} and is principally determined by the high charge density of the ion (*300*). A fairly comprehensive literature exists on its biochemistry. Unfortunately, however, many reports are contradictory since the ion's physical properties have often not been properly taken into consideration (effects have, for instance, been studied under conditions where the amount of Be^{2+} present has exceeded the solubility by several orders of magnitude). Most of the enzymes, reported to be inhibited by Be^{2+}, catalyze reactions where organic phosphate is a substrate, and many also have Mg^{2+} as a normal cofactor (*301,302*). Alkaline phosphatase (*155,301,303,304*) and hexokinase (*301*) can be particularly mentioned, as these are inhibited by Be^{2+} as well as Al^{3+}. ATP binds Be^{2+} (*305*) and several other ATP-ases are also inhibited by it (*301,306,307*).

Compared to Al^{3+}, Be^{2+} is normally present in very small quantities in the environment. The average concentration in the earth's crust is about 10^{-4} of the concentration of Al^{3+}; in lakes and seawater about 10^{-2} (< 1 μg/L, < 1 ng/L, respectively) (*308*). The normal daily intake in food is considered to be in the order of 20 μg (*309*). Be^{2+} is poorly absorbed by the gastrointestinal tract, but some confusion concerning this is present in the literature. In one experiment, Be^{2+} was administered to rats in their drinking water for about 6 months (*310*). Intake and elimination via the urine and feces were measured for each animal, as well as tissue concentrations at the end of the experiment. About 80% of the administered Be^{2+} was recovered in the feces and less than 1% each in the urine and the tissues. Recovery was therefore only about 80%. This was reported and it was most likely due to the chemical method (an isotope technique was not used) underestimating the fecal concentration. The reported primary data therefore have to be recalculated and then show that with 0.16 ppm in the drinking water about 0.8% was absorbed, and with 1.66 ppm about 0.12%. Of the amount Be^{2+} absorbed, 50 to 75% was rapidly excreted in the urine. Unfortunately, however, the experiment has been referred to—even by the author himself (*309*)—as evidence that about 20% of administered Be^{2+} is absorbed. In experiments with ^{7}Be, no uptake ($< 0.2\%$) was detected in rats (*311*) and only 0.3% in calves (*312*).

Life-long administration of Be^{2+} (5 ppm in water) to rats gave no indication of disease, nor shortened the life span (*313*), which also implies a low absorption, since Be^{2+} is markedly toxic with other forms of administration. Moreover, a series of earlier investigations (*309,314,315*) have shown, that with much higher oral doses of Be^{2+}, rickets, due to phosphate deficiency, is the only apparent effect detected.

The literature on the behavior of Be^{2+} in plasma is also somewhat confusing. There are reports that Be^{2+} is present as colloidal Be phosphate (*316,317*), but this is probably true only in situations where comparatively large amounts of Be^{2+} are added artificially. Renal clearance for carrier-free $^{7}Be^{2+}$ is said to be 20 to 40% of the glomerular filtration rate (*318*). A more thorough investigation (*305*) showed that carrier-free $^{7}Be^{2+}$ ($\sim 10^{-9}$ mole/L) is up to about 60% ultrafiltrable in serum. It

was also found that, although Be^{2+} is primarily bound to inorganic phosphate, the complexes are soluble if the Be^{2+} concentration is below 10^{-7} mole/L. In dialyzed serum, Be^{2+} is bound to protein, probably albumin. If physiological concentrations of citrate or phosphate are added to this serum, then Be^{2+} is instead bound to these (*305*). There is no indication that Be^{2+} is ever bound to transferrin.

The distribution of Be^{2+} after IV administration is strongly affected by the amount of Be^{2+} given (*319–323*). A trace amount leaves the blood entirely within minutes, and 35% is excreted in the urine within a few hours. About 50% is bound to the skeleton, where it is presumably very stable (*311,323*), and about 10% is evenly distributed in various tissues (*311,312,319,322*). However, a large amount of Be^{2+}, administered intravenously, leaves the blood more slowly and only 20% is excreted in the urine. In addition, little is bound to the skeleton but a great deal is bound by the liver and also by the spleen (*319*). After oral administration, Be^{2+} is primarily bound to the skeleton (*310,312*) i.e., as after IV injection of trace amounts.

The distribution and effects of IV-administered colloidal Be phosphate has also been reported in several articles (*324*). Be-phosphate is rapidly (< 1 hr) taken up by the liver, initially almost solely by the Kupffer cells (*325,326*) that therefore degenerate and die within 24 hr.

If, instead, Be^{2+} is administered as a sulfosalicylate complex (*327*) that prevents the formation of colloidal Be hydroxide and Be phosphate, the distribution is changed so that more is excreted in the urine and more is bound by the skeleton, but less by the liver and spleen. However, the toxicity increases, due probably to the parenchymal cells of the liver now being affected, as well as the cells of many other organs. It is also noticeable how T-lymphocytes in the spleen, thymus, and lymph nodes are strongly affected, while B-lymphocytes in the bone marrow are comparatively unaffected. *In vitro* studies have confirmed that macrophages easily take up Be phosphate and Be hydroxide, and to a much less extent take up soluble Be complexes.

Investigations of the cell metabolism of Be^{2+} have shown that Be^{2+} phosphate and hydroxide are first taken up by the lysosomes (*328*) and that Be^{2+} is then transferred to the cell nucleus (*321,329*). According to earlier reports, this occurs within 24 hr, and according to more recent reports this occurs only after 2 to 3 weeks (*330*). In the nucleus Be^{2+} is bound to chromatin and it has shown a greater affinity to intact cell nuclei than to an equivalent amount of DNA, which has been taken to indicate that it is not the DNA, itself, that binds Be^{2+} (*302,321,329*). However, the experiments were performed in the presence of excess citrate or sulfosalicylate to prevent precipitation of hydroxide. They can therefore also be interpreted to indicate that intact nuclei—with a high local concentration of DNA with a native tertiary structure—can compete better with other complex-forming agents for Be^{2+} than DNA in simple solutions. Under other conditions, Be^{2+} is bound directly to DNA and affects its structure (*331*).

The true reason for cell death by Be^{2+} poisoning has been debated. One possibility is that the lysosomes disintegrate (*332*), another that vital enzymes are inhibited (*333*). Be^{2+} is said to inhibit DNA synthesis (*334,335*) and RNA synthesis (*336*) and has also been reported to cause a reduced fidelity in DNA synthesis *in vitro* (*337,338*).

A large number of investigations have unambiguously shown that Be^{2+} can induce cancer in experimental animals (*339–342*), but there is no definite epidemiological evidence that the present-day industrial exposure has produced cancer in humans (*341*). Mutagenicity tests with microorganisms have generally shown negative results (*342*).

In humans and various experimental animals, Be^{2+} causes cell-mediated delayed hypersensitivity (type IV allergy), but only with skin exposition or inhalation. Intravenous, intraperitoneal, or oral administration (guinea pig) instead causes tolerance that prevents hypersensitivity (*343,344*). The generally accepted opinion is that Be^{2+}–protein complexes and not the ion itself trigger the hypersensitivity and cause the symptoms. In humans, Be^{2+} hypersensitivity has primarily caused contact dermatitis, mucous membrane symptoms (e.g., conjunctivitis), and a chronic lung granulomatosis called berylliosis. Skin and lung manifestations have often occurred together.

Zirconium. The literature concerning the biochemistry of Zr^{4+} is very limited in view of the relative abundance of the ion in the natural environment (Table 1). The probable explanation is that Zr^{4+} compounds are considered to be very atoxic, due mostly to their low solubility (*345*). Zr^{4+} is reported to inhibit alkaline phosphatase (*346*) and ATPase (*299*).

Three technical applications illustrate the biological similarities of Zr^{4+} and Al^{3+}. Zr^{4+} is used, to some extent, as an antiperspirant and has an advantage over Al^{3+} as it has a longer-lasting effect. Further, Zr^{4+} is used for hide tanning, as is also Cr^{3+} and, in the past, Al^{3+} (*347*). Finally, Zr^{4+} has been used as a mordant for histological staining with hematoxylin (*348*) (as an alternative to Al^{3+}, Cr^{3+} or Fe^{3+}). Histologically, Zr^{4+} is mostly bound to the cell nuclei and glycosaminoglycans. The latter binding has also been used in a quantitative method to determine these substances (*349*).

In the gastrointestinal tract, the uptake of the ion is probably minimal (*299*). However, Zr^{4+} is normally detectable in tissues and the only published investigation in humans showed a wide range of values, probably due to technical reasons. Excluding some very high values, all from single determinations, the highest concentrations found were those in the liver, lung and kidney (the skeleton was not investigated) (*350*). The total body content was estimated to be about 290 mg, but the primary data for this estimation appear to be inconclusive. In mice, after 1 to 2 years of experimental exposition to Zr^{4+} in drinking water, the greatest increase in tissue concentrations was observed in the kidney and spleen (*351*). No toxic effects were observed.

^{95}Zr hydroxylacetate administered intravenously to

rabbits was eliminated from the blood with a half-life of about 70 min (*352*). A few percent was excreted in the urine and, of the remaining amount, 44% was bound to the skeleton and 37% in the liver. A high tissue uptake per weight was also noted in the spleen and bone marrow.

Zr^{4+} has earlier been used in various dermatological remedies. The introduction of Zr^{4+} in deodorants in the middle of the 1950s led, however, to immediate reports of a new type of local granulomatosis in the axillae, which was evidently caused by Zr^{4+} (*345*). As this effect was induced only in some users, it was immediately accepted as being an allergic manifestation. The granuloma was very similar to the Be granuloma, that was also of interest. Similar changes could, however, also be induced in humans as a foreign body reaction after intracutaneous injections of Zr^{4+} (*353,354*). Animal experiments (rabbit) have later confirmed that granulomas can be induced with ease, but that it is difficult to prove these are allergic reactions (*355,356*). However, guinea pigs are reported to produce a genuine hypersensitivity that also showed a cross-reactivity with Cr^{3+} (*357*). In a large investigation in humans, hypersensitivity was detected in 1 to 2% of the individuals six months after intracutaneous injection of Zr^{4+} (*358*). The similarity between the histological changes induced by Zr^{4+} and Be^{2+}, together with the fact that deodorants with Zr^{4+} have also been used as aerosols, have given rise to several investigations on the effect of Zr^{4+} after inhalation (*298,359,360*). However, investigations performed with rats and hamsters showed only toxic effects, and no condition similar to berylliosis was induced; nor has any lung granulomatosis, produced by Zr^{4+}, been detected in humans.

Chromium. In many respects, Cr^{3+} is chemically similar to Al^{3+} but is even more prone to polymerize in neutral aqueous solutions and to form very stable coordination complexes (see note 4). Cr^{3+} complexes with ATP and other nucleotides, including several isomeric forms (*361*), are noteworthy among the various complexes. Most Cr^{3+}–nucleotide complexes, more or less, inhibit enzyme reactions that are dependent on the corresponding Mg^{2+} complexes. Cr^{3+}–ATP has, therefore, been used to study reaction kinetics, particularly for various kinases, e.g., hexokinase (*362*). Cr^{3+} also inhibits $Na^+ + K^+$ – ATPase (*363*), Ca^{2+}– and Mg^{2+}–ATPase (*364,365*), and alkaline phosphatase (*146*) but is stated, as with Al^{3+}, to activate trypsin (*170*), phosphoglucomutase (*167*) and succinic dehydrogenase (*168*).

Cr^{3+} is strongly bound to RNA, DNA, and various proteins (*366*), whereby Cr^{3+} ions or Cr^{3+} oligomers form crosslinks between different chains. An important technical application of the protein binding is the Cr^{3+} tannage, that has now replaced Al^{3+} tannage (*367*). Cr^{3+} gives leather a better water resistance than Al^{3+} due to the higher stability of the Cr^{3+} complexes. The binding of Cr^{3+} to DNA has not been studied in detail, but according to some reports, binding occurs only to the phosphate groups (*174,368*) as (probably) with Al^{3+}. The binding causes a condensation of DNA (*369*).

Trace amounts of Cr^{3+} are found in many minerals, where Cr^{3+} replaces Al^{3+} or Fe^{3+}. Cr^{3+} is, therefore, a very ubiquitous ion despite its average concentration in the earth's crust being only 0.1%, by weight, of that of Al^{3+}. The Cr^{3+} concentration in lakes and rivers is 1 to 10 μg/L (or greater) but lower in seawater, 0.05 to 0.5 μg/L, similar to Al^{3+} (*366*). Small amounts of Cr^{3+} are taken up by plants but most of it remains in the roots; Cr^{3+} has no function in higher plant species (*370*). Very high amounts of Cr^{3+} in the soil are considered to inhibit plant growth but, at the same time, it has been reported that with the addition of Cr^{3+} to Cr-poor soils a considerable growth increase is noted for various crops (*366*). Possible reasons could be that microorganisms in the soil require Cr^{3+}, and thus the higher plants are indirectly stimulated or that other ions, essential for the plants but strongly bound in the soil, are displaced by Cr^{3+} and released.

Due to Cr^{3+} uptake in crops, most foods contain Cr^{3+} in an amount corresponding to 0.1 to 10%, by weight, of the Al^{3+} amount. Intake of Cr^{3+} in the Western World is reported to be about 5 to 100 μg/day (*17,371–374*), which is equivalent to 0.5% (molarly 0.26%) of the supposed Al^{3+} intake (1 to 20 mg/day).

The absorption of Cr^{3+} in the gastrointestinal tract is believed to be strongly influenced by the type of compounds ingested or formed in the intestine (*366*). When administered as chloride, only 0.1 to 1% is absorbed (*375–378*), but organic complexes are believed to be taken up to a considerably larger extent (*366,377,379*). Earlier it was believed that at least 10% of all Cr^{3+} ingested was absorbed (*373,375,380*), which, therefore, indicated that much Cr^{3+} in food existed as firm complexes. This conclusion was based on comparison of the above stated normal intake of Cr^{3+} and the normal urinary excretion, which was reported to be 5 to 10 μg/day (*381,382*). Recent determinations have, however, shown that the latter value is probably about 10 times in excess of the true value (*383–391*). If the lower value is accepted as being correct, this implies that the urinary excretion of Cr^{3+} on a molar basis is 1 to 2% of the excreted amount of Al^{3+} and that about 1% of the Cr^{3+} intake is absorbed.

According to reports, Cr^{3+} is 90 to 95% transferrin-bound in blood plasma (*392,393*), and probably is bound in the same manner as Fe^{3+} (*394*). The remainder is ultrafiltrable and very likely bound to various complex-forming small molecules. Information regarding the normal concentration in serum, as with Al^{3+}, is contradictory (*366,395–397*), but recent values of about 0.15 μg/L are most plausible (*381,395*). In that case, the concentration corresponds to 2 to 3% of the supposed molar concentration of Al^{3+}.

The total body content of Cr^{3+} is low and is reported to be below 6 mg (*398*). According to another report (*21*) discussed in detail below, one can assume that the total body content of Cr^{3+}, on a molar basis, is about 3% of the value for Al^{3+}. This would imply a body content of about 2 mg. The distribution in the various organs closely compares with the distribution of Al^{3+} (*21*). A recent and comprehensive review on tissue occurrence and distribution of Cr^{3+} is given elsewhere (*397*).

Contradictory information is reported in the literature

concerning the effects of age on tissue concentrations of Cr^{3+}. The concentrations are reported to be comparatively high in fetuses and newborns but are reported to decrease, thereafter (*287,371,377*). However, the grounds for these claims appear to be weak, although the reports have often been cited. A previous investigation regarding the occurrence of Cr^{3+} in the tissues of humans and animals (and various foods) (*371*) has also often been referred to as evidence that "chromium deficiency" is a danger threatening old people (*366*). The primary data in this investigation varied so much that only contamination can explain the reported values. A recent investigation showed increased concentrations of Cr^{3+} with increasing age in the cerebral cortex and basal ganglia (*287*), but the total material studied consisted of only six individuals. Cr^{3+} that is deposited in the lungs of people exposed to chromium in the air, is believed to remain stationary for the rest of their lives (*399*).

The distribution of IV-administered Cr^{3+} in the body is mainly dependent on the quantity given and on its chemical form. When large amounts are given, colloids are formed, most of which are taken up by the reticuloendothelial system (chiefly in the liver); comparatively small amounts are bound to the skeleton or excreted in the urine (*376*). If only a trace amount is administered, the main part is excreted by the kidneys, and the highest uptake is reported in the skeleton (*400*), where Cr^{3+} binds primarily to the epiphyseal plates (*401*). A similar distribution is obtained if Cr^{3+} is given as a complex, e.g., with acetate or citrate (*376,401*). If the complexes are even more stable, e.g., Cr-EDTA, renal excretion is total. Independent of the mode of administration, however, shortly after the injection a high uptake is seen in the lungs and kidneys (*400–403*). The lowest uptake is reported in the brain and muscle tissue (*402*).

Few investigations seem to have been reported on the fate of tissue bound Cr^{3+}. In one investigation, about 1 μg $^{51}Cr^{3+}$ was administered intravenously to healthy volunteers, and the retention was followed by whole body counting (*404*). The data indicated the existence of three compartments with biological half-lives of 0.56, 13.2, and 195 days, respectively. The latter pool represented about 60% of the given dose. This pool was followed only a little more than one biological half-life (but almost 10 physical). Therefore, it can be assumed possible that compartments exist with considerably longer biological half-lives. Later studies by the same group gave similar results (*405*). One investigation (*392*) performed with rats suggested that 30 to 40% of the administered dose was bound, apparently irreversibly. Another study (*406*) proposed the presence of three Cr^{3+}-binding compartments, and suggested that the fraction eliminated most slowly comprised about 27% of the given dose and had a biological half-life of 83 days (uncertainty was, however, in both cases high as the observation period was short).

Cr^{3+} is transferred very slowly into the cells, and the mechanisms governing its uptake have not been investigated. How Cr^{3+} is metabolized intracellularly is also uncertain. In short-term trials, after administration of Cr(VI), intracellular Cr has mainly been localized to the cytosol probably as complexes with comparatively small molecules (*402,407,408*). An accumulation in the cell nuclei occurs, however, after *in vivo* administration of Cr^{3+}; in liver cells, about half of all the Cr^{3+} was found in the cell nuclei (*409–411*). As mentioned above, Cr^{3+} is bound to RNA and DNA. The binding to DNA has been thought to serve as a sink for the ion and also to reduce the solubility and stabilize the double-strand structure of DNA (*412*). Cr^{3+} is also reported to interfere with the DNA and RNA synthesis (*409*) and with the cell mitosis. It has been shown to induce a dose-dependent increase of sister chromatid exchanges in hamsters (*413*).

Cr^{3+} is generally assumed to remain in the cells for the rest of their life, and this is the basis for several cytotoxicity tests, where cells are labeled with $^{51}Cr^{3+}$ and leakage of the radionuclide is interpreted as a sign of cell destruction.

Cr is carcinogenic, but recent epidemiological investigations indicate that only Cr(VI) exposition involves a cancer risk (*415,416*). This risk has given rise to careful studies also of Cr^{3+} to detect any potential carcinogenicity (*417*). Cr^{3+} has been reported to reduce the fidelity and rate of DNA synthesis *in vitro*, with viral, bacterial, or human DNA polymerase (*177,418*), but the concentration used was very high, 0.1 to 5 mmole/L, well above the ion's maximal solubility. In most cases, no effects have been observed in mutagenicity tests with various microorganisms when Cr^{3+} has been added in the form of inorganic salts (*417,419*), but Cr^{3+} in weak complexes with acetate (*417*), citrate, or salicylate (*420*) had weak mutagenic effects, far weaker than Cr(VI). Stronger and perhaps more lipophilic chelates with bipyridine or 1,10-phenanthroline showed, however, a considerable mutagenicity in several different test systems (*421*). Cr^{3+} is also reported to interfere with mitosis and to induce chromosomal damage in different tumor-cell lines *in vitro* (*422*), but the investigation was performed with an extremely high Cr^{3+} concentration (about 10 mmole/L, 3-4 orders of magnitude above the solubility limit); this also caused severe cytotoxic effects. Another investigation with normal human leukocytes (*423*) and a lower Cr^{3+} concentration showed a barely significant increase in the frequency of chromosomal breakage with Cr^{3+} acetate in a concentration of at least 16 μmole/L but not with Cr^{3+} nitrate or chloride. Other similar investigations have been entirely negative (*415*).

In the previously mentioned investigations, much stronger effects were induced by Cr(VI) than by Cr^{3+}. Evidence indicates that the reason is that Cr(VI), contrary to Cr^{3+}, is easily taken up by the cells. Once inside, Cr(VI) is immediately reduced to Cr^{3+}. This Cr^{3+} will be located in the cytosol (while any Cr^{3+} taken up as such, probably will be confined in endocytotic vesicles or lysosomes). It is, therefore, more easily bound to DNA and, perhaps, can cause the mutations and chromosomal damage that induce cancer (*417,424–426*). Another possible mechanism could be that the actual reduction of Cr(VI) leads to oxidation of other cell structures making them carcinogenic (*415*). This latter hypothesis apparently lacks experimental support, while the general prop-

erties and behavior of Cr^{3+} in the cells seem to make the former hypothesis highly plausible. If Cr^{3+} is the final mutagen and carcinogen, then Cr(VI) can be said to have been essential, in short-term experiments, in disclosing the mutagenic and clastogenic properties of Cr^{3+}. If Cr(VI) had not existed, we would presumably not have discovered the very slow effects of Cr^{3+}.

Cr is one of the most common causes of contact dermatitis, and it is almost entirely Cr(VI) exposure that triggers the hypersensitivity and produces the symptoms. Exposure to Cr^{3+} alone most likely rarely induces hypersensitivity and only causes slight symptoms in a person already sensitized through Cr(VI).

Agreement exists in the literature that the immunologic reaction in Cr hypersensitivity is always induced by a complex of Cr^{3+} and some macromolecular carrier, usually a protein (*428*). However, it is difficult for Cr^{3+} to enter the skin and reach the level where immunologic reactions can take place. Therefore, Cr^{3+} has difficulties in both sensitizing and producing symptoms. Cr(VI) can, on the other hand, enter the skin with ease, then be reduced to Cr^{3+} and subsequently be bound to the appropriate carrier, thus causing hypersensitivity and dermatitis. Guinea pigs with experimentally induced Cr hypersensitivity can be desensitized by intravenous injections of Cr(VI); but the amount necessary is in the same range as LD_{50} (*428*). No known method exists for desensitizing humans suffering from Cr allergy.

Long-term inhalation of small amounts of Cr(VI) produces conditions resembling bronchitis and asthma (*428*), but no agreement has been reached as to whether the conditions should be classified as toxic or allergic. Lung affections of the pneumoconiosis type have also been reported (*380*).

In the literature concerning nutrition, Cr^{3+} is generally stated to be an essential trace element but its only known function is its asserted role in a hypothetical glucose tolerance factor, GTF. This factor is claimed to be most abundant in, for example, Brewer's yeast and pig kidney and is assumed to contain niacin in addition to Cr^{3+}. GTF has not been isolated, nor has its structure been determined. Recently, one group even claimed that they had separated the biological activity from the Cr^{3+}-containing material (*429*). The extensive literature has been surveyed in several reviews (*366,430–432*). The various findings indicating the existence of GTF and the role of Cr^{3+} in it can be assigned to one of the following groups.

Experimental animals fed a special Cr^{3+}-poor diet are reported to develop a decreased glucose tolerance which could be prevented by addition of various Cr^{3+} compounds, particularly organic complexes (*366,432,433*). However, the reported effects have been small, and later workers have not succeeded in producing significant effects (*434*).

Some patients with diabetes are claimed to have improved after administration of Cr^{3+} or yeast extract (*432*). The studied groups have, however, always been small (often less than 10 individuals), and the reported effects have also been limited. The investigations have generally been carried out poorly, very often with the omission of control groups, and they have occasionally used objectionable statistical manipulations (see note 5). Three double-blind investigations showed no effect (*435–437*).

Glucose and insulin are both claimed to cause increased serum concentration and urinary excretion of Cr^{3+}. This effect is also said to be lacking in many diabetics, and lack of an increase of Cr^{3+} during a glucose tolerance test should, therefore, be the best method of demonstrating Cr^{3+} deficiency (*432*). Other investigations have, however, shown a reduction in serum and urine concentrations of Cr^{3+} after glucose administration (*438*). All the above investigations have been performed with assay techniques resulting in 10 to 100 times higher values than are now accepted as being accurate.

It is claimed that insulin-dependent diabetics absorb considerably more orally administered $^{51}Cr^{3+}$ than healthy individuals or noninsulin-dependent diabetics and also excrete more of the intravenously administered $^{51}Cr^{3+}$ in the urine. The increased urinary excretion was supposed to be primary and to have induced a Cr^{3+} deficiency and a compensatory increase of the intestinal uptake (*396*). Another report claimed that diabetics absorbed $^{51}Cr^{3+}$ considerably better than normal individuals while at the same time, Cr^{3+} was excreted less in the urine (*439*), findings that, according to the authors, indicated a large Cr^{3+} deficiency in the tissues. Conflicting results regarding serum concentration of Cr^{3+} in diabetics have been reported. Some groups have found markedly increased serum concentrations (*382,385*) with increased urinary excretion. One author has reported decreased concentrations in younger diabetics but increased in older (*428*), and one group found no correlation between the Cr^{3+} concentration and the glucose tolerance (*440*).

Cr^{3+} is reported to potentiate the effect of insulin on the tissue uptake of glucose. *In vitro* glucose (*441*) or galactose (*442*) uptake in fatty tissue was thus said to increase 25 to 75% when Cr^{3+} was added, on the condition that the tissue source was from animals raised on a GTF-deficient diet. Similar experiments performed with isolated adipocytes and various organic Cr^{3+} complexes showed a potentiation of over 300% (*443*).

The literature regarding GTF and Cr^{3+} is extremely difficult to assess, and even supporters of the GTF hypothesis seem to be unable to state which article or articles finally established Cr^{3+} as an essential trace element. Most reports that support the role of Cr^{3+} in GTF originate directly or indirectly from one group of workers. Despite the passing of 25 years since the first reports were published, the group has evidently not succeeded in convincing the majority of the insulin and diabetes investigators. Cr^{3+} and GTF are hardly mentioned in recent reviews concerning insulin and insulin receptors. Moreover, the literature regarding GTF has almost entirely been published in other than the usual endocrinological and diabetological journals.

Iron. In spite of many similarities between Fe^{3+} and Al^{3+}, their metabolisms are very different (see Tables 6

Table 6. Comparison of the normal metabolism for Fe^{3+} and Al^{3+} in adults.[a]

	Fe^{3+}	Al^{3+}	Fe/Al ratio
Normal dietary contents, μmole/day	200–300	40–750	approx. 1 (0.1–10)
Daily uptake, μmole/day	20	1.5	13
Body contents, mmole	70	1.5	45
Plasma, transferrin bound, μmole/L	20	0.1	200
Plasma, other forms, nmole/L	0.05	10	0.005
Biological half-life in plasma, min	165	250	
Urinary excretion, μmole/day	2[b]	1	2
Milk concentration, μmole/L	7[c]	13	0.5

[a] Information regarding Fe is obtained from standard works in Clinical Chemistry unless otherwise noted. References regarding Al are given in the discussion.
[b] According to the literature (*444*).
[c] According to the literature (*445*).

and 7). The main reasons for the differences are the ability of Fe^{3+} to be reduced to Fe^{2+} and the high specificity of the Fe binding proteins, transferrin and ferritin. Some aspects where the ions differ are briefly commented on below, but Fe^{3+} metabolism is otherwise omitted.

At the current redox-potential in the cytosol the metabolically active Fe pool exists mainly as Fe^{2+} (*447*), presumably as complexes with, e.g., amino acids. Fe^{2+} is far less inclined than Fe^{3+} and Al^{3+} to form irreversible complexes with proteins and other macromolecules. However, ferritin takes up Fe^{2+}, oxidizes it to Fe^{3+} (*448*), and binds it in the central cavity of the molecule as hydroxide. In principle, this binding would probably be very firm, but Fe^{3+} can be reduced and liberated if ferritin is taken up by the lysosomes, as their low pH facilitates the reduction of Fe^{3+}. Ferritin Fe^{3+} is, therefore, potentially much more metabolically active than any Al^{3+} that has formed, more or less, irreversible complexes with other cell structures. There is no evidence that Al^{3+} also binds to ferritin (but possibly this has not been investigated).

Transferrin totally determines the extracellular metabolism of Fe^{3+}. The binding constant is so high that only an extremely small fraction is ultrafiltrable. This largely prevents loss in urine. The number of transferrin receptors on various cells control the distribution of Fe^{3+} to them. The transport is directed from the intestinal mucosa towards the other tissues; as mucosal cell receptors chiefly bind Fe^{3+}-free transferrin and receptors on other cells bind the Fe^{3+}–transferrin complex. Despite the low solubility of Fe^{3+}, its metabolism is very lively and most body cells are thought to be in a state of Fe^{3+} equilibrium.

Table 7. Molar ratio of Fe^{3+}/Al^{3+} in various organs of healthy individuals.[a]

Organ	Fe^{3+}/Al^{3+}	Organ	Fe^{3+}/Al^{3+}
Spleen	424	Testis	34
Liver	144	Urinary bladder	32
Kidney	123	Esophagus	32
Heart	113	Omentum	29
Brain	103	Thyroid	26
Muscle	102	Prostate	25
Diaphragm	81	Trachea	22
Pancreas	66	Rectum	21
Ovary	57	Colon	18
Stomach	51	Ileum	18
Duodenum	50	Adrenal	17
Aorta	45	Cecum	12
Jejunum	40	Lung	6.5
Uterus	39	Skin	3.2
Larynx	38	Hair[b]	1.35

[a] Calculated from literature data (*21*) unless otherwise noted.
[b] According to ref. (*446*).

As seen in Table 7, the Fe/Al ratio in different organs varies within two orders of magnitude. High ratios are reported in the spleen, liver, and kidney and are mainly due to high Fe concentrations; high ratios in the heart, brain, muscle, and diaphragm are, however, due to low Al^{3+} concentrations. Low ratios in the intestine, lung, skin, and hair are mainly caused by the relatively high Al^{3+} concentrations in these tissues.

The intestinal uptake of Fe is much greater than the uptake of Al^{3+}. This may partly be due to the occurrence of heme–Fe and inorganic Fe^{2+} in the intestine; both are easily taken up by the mucosal cells better than Fe^{3+} and probably also better than Al^{3+}. The acid environment in the stomach and the probable reducing environment in the intestine both favor reduction of Fe^{3+} to Fe^{2+}. The absorption is also increased by the presence of complexing substances, e.g., citrate or fructose, which possibly hints at Fe^{3+} also being absorbed. Fe uptake is believed to be regulated to a certain degree by the needs of the body. An abundance of Fe in the mucosal cells possibly increases their synthesis of ferritin. A larger proportion of the Fe taken up by the cells is then bound and returned to the intestinal lumen when the cells die. Probably no similar regulation of Al^{3+} absorption exists.

The chemical similarities between Fe^{3+} and Al^{3+} are most prominent in the metabolism associated with chronic Fe^{3+} overloading, hemochromatosis. This condition appears in two entirely different forms, namely idiopathic (or primary) hemochromatosis (IH) and secondary hemochromatosis. The former is caused by a hereditary tendency to absorb too much Fe in the intestine, the latter by an excess supply of Fe in food or by repeated blood transfusions. The iron distribution in the body differs in the two forms. In secondary hemochromatosis, Fe^{3+} is found mainly in the macrophages of the reticuloendothelial system, but in IH Fe^{3+} is reported to be low in these cells as it is in the intestinal mucosal cells. Instead, Fe^{3+} is deposited in the hepatocytes, in various endocrine glands, in the skin, the skeleton, the heart, and occasionally also in the skeletal muscles.

General Discussion on Metabolism and Effects of Al^{3+}

Comparison between the Various Ions. The above review of the literature shows that the knowledge in this field is still fragmentary. Therefore, a thoroughly systematic and detailed comparison is impossible. Clearly, however, the literature shows a large number of striking similarities between the various ions in most of the hitherto

studied aspects and it shows only a few examples of definite differences.

ORGAN DISTRIBUTION: Most remarkable are the similarities between the patterns of occurrence in various organs revealed by the Al^{3+}-like ions. Two fairly detailed investigations have been published regarding the normal concentration of Al^{3+} and other trace elements in human tissues (persons that had died after accidents) (*21,22*). Due to differences in choice of tissues, in handling of the samples and in assay methods the two investigations are not directly comparable, but the distributions of Al^{3+} in the organs reported by the two articles are in general agreement. Moreover, one of the investigations (*22*) showed very similar distributions in eight different organs of Al^{3+}, Ga^{3+}, Cr^{3+}, Y^{3+}, and Zr^{4+} (Table 8), and the other investigation (*21*) showed a close correlation ($r = 0.96$) between Al^{3+} and Cr^{3+} in 28 different organs (see Fig. 2 and note 6). However, one organ, the lung, differed considerably. From data in this investigation (*21*), the average Cr/Al molar ratio for all the other organs can be estimated to about 3%. In the lung, the concentration of both ions was very high and the Cr/Al molar ratio was 0.65%, i.e., considerably closer to the Cr/Al ratio of the earth's crust (0.06%); which probably means that Cr^{3+} and Al^{3+} in the lungs had entered mostly via the respiratory tracts. According to the other investigation (*22*), the Cr/Al ratio varied between 1 and 3%. A third investigation indicates that a correlation may also exist between the concentration of Cr^{3+} and Sc^{3+} in the brain of different individuals (*287*).

The credibility and importance of these correlations increases when considering that the normal Cr/Al molar ratio can be calculated to 2 to 3% for plasma, and 1 to 2% for urine (see discussion above). In addition, other investigations that have included Al^{3+} and Cr^{3+} in normal tissues seem to show average Cr/Al molar ratios of the same magnitude; namely 1.4% (*451*) and 2.7% (*446*) for hair, 2.4% (*449*) for nails, and 0.88% (*450*) for the liver.

The correlations are very remarkable, as they seem to indicate that all organs probably treat the ions very similarly (at least Al^{3+} and Cr^{3+}) both in regard to the uptake and elimination from the tissues. These similarities have not previously been observed, and apparently not even the authors of the quoted works (*21,22*) have observed the correlations—nothing is, at any rate, mentioned in their articles.

If the d^3-ion Cr^{3+} and the "inert gas ion" Al^{3+} are similarly metabolized, then there is reason to believe that the metabolisms of the "inert gas ion" Sc^{3+} and the d^{10}-ion Ga^{3+} can show just as many similarities to Al^{3+} metabolism. Radionuclides of all three, Cr^{3+}, Sc^{3+}, and Ga^{3+}, could then be used as substitutes in investigations regarding the metabolism of Al^{3+}, just as discussed recently (*452*) and as has already been done in plant physiology (*278*).

TOXICITY: Among the few quantitative data on the metabolism of the ions being compared, the data concerning their acute toxicity after parenteral administration are worth special mention. In Table 9, available LD_{50} values for intravenous or intraperitoneal administration are given. Great differences exist in the modes of investigation and the way the findings are reported, which undoubtedly makes detailed comparisons of the values from the different investigations quite impossible. If some values that represent fairly stable chelates with low toxicity are omitted, it is evident that most of the values for Al^{3+}, Sc^{3+}, Y^{3+}, and Cr^{3+} seem to be grouped around the value of 1 mmole/kg body weight, whereas Ga^{3+} shows roughly a fivefold lower value and In^{3+} roughly an additionally tenfold lower value. No definite conclusions regarding Zr^{4+} can be made, a couple of values are reported at the same level as Al^{3+}, others are considerably higher.

Descriptions of how the experimental animals died are rather scanty and therefore it is not possible to definitely determine if the clustering of the values around 1 mmole/kg is purely accidental or if it is a result of a threshold value for some common toxicological mechanism. In cases when the various salts were injected intravenously the concentration, after mixing in the circulating blood, should have given plasma values of about 25 mmole/L. This concentration is roughly three orders of magnitude above the free binding capacity of transferrin in plasma. Exceeding that cannot, therefore, be the toxicological threshold. As the ions probably rapidly hydrolyze after injection, the amount given could also be compared with, for example, 75 mmole acetic acid per liter plasma. With this in mind, the LD_{50} values must be considered remarkably high. Possibly, the immediate acidifying effect may have been a factor contributing to the death of many animals and thereby helping to create very similar LD_{50} values.

Table 8. Occurrence of certain elements (average values) in healthy human tissues (*22*)[a].

	Concn, μg/kg wet weight				
	Al^{3+}	Ga^{3+}	Cr^{3+}	Zr^{4+}	Y^{3+}
Brain	200	0.6	10	20	7
Kidney	400	0.9	30	20	6
Liver	2600	0.7	80	30	10
Lung	18,200	5	500	60	20
Lymph node	32,500	7	2200	300	60
Muscle	500	0.3	5	20	4
Testis	400	0.9	30	10	4
Ovary	400	2	60	30	10

[a] Concentrations have been determined by mass spectrometry or by X-ray fluorescence and are given in μg/kg wet weight.

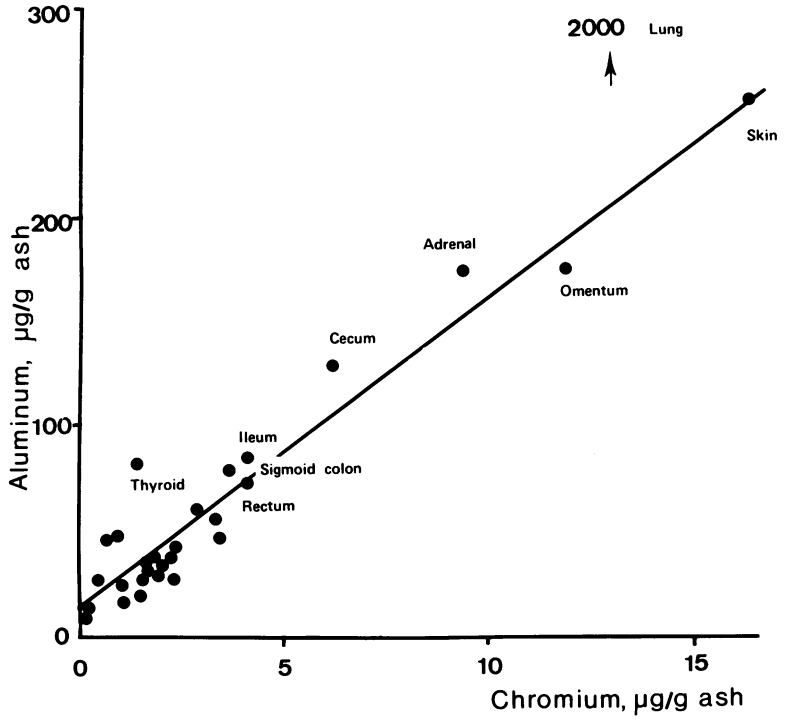

FIGURE 2. Concentrations of Al^{3+} and Cr^{3+} in different tissues from healthy individuals. The values are derived from the literature (*21*), and each recorded point represents the median value for one specific tissue obtained from 150 individuals. The analyses were performed by emission spectroscopy and the tissues were those given in Table 7.

On applying the animal experimental value of 1 mmole/kg to humans, the LD_{50} for adult men should be about 70 mmole or almost 2 g. After chronic hemodialysis with water containing too much Al^{3+}, death has been reported to occur with a total accumulated amount (= administered dose, since apparently no elimination occurs) of maximally 3 g, according to a method utilizing *in vivo* whole-body neutron activation (*461*). The value is also well in agreement with chemical determinations (*23*). If the agreement with the animal experimental values is not accidental, it suggests a somewhat more specific toxicological mechanism than the "acid effect" discussed above since this can be excluded in dialysis.

No explanation has been found in the literature for the greater toxicities of Ga^{3+} and In^{3+} compared to the other trivalent ions, but some metabolic observations could, together with differences in the chemical properties, indicate a possible mechanism.

In^{3+} is apparently metabolized in different ways depending on the mode of administration as a soluble salt or as a precipitated hydroxide (*260*). The salt is eliminated more rapidly and mainly via the kidneys; the hydroxide slower and mainly via the gastrointestinal tract. The hydroxide was also more toxic than the salt solution (see Table 9). Much evidence supports insoluble metal hydroxides being mainly taken up by the reticuloendothelial cells, while soluble salts of the trivalent ions are mainly bound to the skeleton or excreted in the urine. The higher toxicity of In^{3+} hydroxide compared to soluble In^{3+} salts could therefore, hypothetically, be due to the hydroxide being taken up by the reticuloendothelial cells, thereby affecting a comparatively small cell mass.

At a neutral pH the solubility of In^{3+} is at least one order of magnitude lower than the solubility of Al^{3+}, Ga^{3+}, Sc^{3+}, Y^{3+}, and Be^{2+} and several orders of magnitude lower than that of Cr^{3+} (see Fig. 1). As hydrolysis of the added ions tends to lower the pH, the range immediately below the neutral point is also of interest. In this range both Ga^{3+} and In^{3+} have lower solubilities than the other ions. For example, at pH 5 their solubility is two orders of magnitude lower than for Al^{3+} and more than four orders of magnitude lower than for the other ions. Ga^{3+} and In^{3+} should, therefore, in this particular pH range, form colloids at a 10 to 100 times lower concentration than the other trivalent ions and thereby be metabolized more easily via the more dangerous route.

Even if the reported LD_{50} values for Be^{2+} and In^{3+} are very similar their toxicological mechanisms probably differ. Dissolved Be^{2+} given intravenously, is thus reported to be more toxic than insoluble Be-phosphate (*458*). The difference compared to In^{3+} could probably be due to dissolved Be^{2+} not being metabolized via transferrin and the lysosomes and being more easily taken up by the cell cytosol than the trivalent ions. Be^{2+} is relatively soluble at pH 5 to 7 (see Fig. 1), which reduces the risk for colloid formation and favors the more toxic soluble form.

Both Fe^{3+} and Zr^{4+} have extremely low solubilities within the entire relevant pH range, but are still considered to be moderately toxic. This might be due to the intracellular metabolism. Fe^{3+} is neutralized by ferritin and Zr^{4+} has an extremely low solubility even at the pH

Table 9. LD_{50} with intravenous (IV) or intraperitoneal (IP) administration of Al^{3+} or similar ions according to reports in the literature.

Cation	LD_{50}, mmole/kg	Mode of administration	Species	Reference
Al^{3+}	1.2	IV	Rabbit	(*453*)
	0.85	IP	Mouse	(*274*)
	0.87	IP	Rat	(*274*)
	0.80	IP	Mouse	(*454*)
Ga^{3+}	0.18	IP	Mouse	(*274*)
	0.15	IP	Rat	(*274*)
	0.21	IP	Mouse	(*454*)
	0.66[a]	IV	Rat	(*211*)
In^{3+}	0.016	IP	Mouse	(*274*)
	0.012	IP	Rat	(*274*)
	0.022	IP	Mouse	(*454*)
	0.11	IV	Mouse	(*260*)
	0.0028[b]	IV	Mouse	(*260*)
Sc^{3+}	2.9	IP	Mouse	(*455*)
	1.6	IP	Mouse	(*283*)
	0.09	IV	Mouse	(*283*)
	0.62	IP	Mouse	(*454*)
Y^{3+}	1.4	IP	Rat	(*299*)
	0.66	IP	Mouse	(*454*)
Zr^{4+}	0.7–10.3[c]	IP	Rat	(*299*)
	2.7[a]	IP	Rat	(*456*)
	18.6[a]	IP	Rat	(*456*)
	0.96	IP	Mouse	(*454*)
Be^{2+}	0.031	IV	Rat	(*327*)
	0.178[a]	IV	Rat	(*327*)
	0.049	IV	Rat	(*333*)
	0.057	IV	Rat	(*316*)
	0.016	IV	Mouse	(*457*)
	0.026	IV	Rat	(*457*)
	0.156[b]	IV	Rat	(*457*)
	0.029	IV	Mouse	(*458*)
	0.15	IP	Mouse	(*454*)
Cr^{3+}	0.60–1.1[c,d]	IV	Rabbit	(*459*)
	0.80–3.0[c,d]	IV	Mouse	(*459*)
	0.90	IP	Mouse	(*454*)
	0.15–0.45	IP	Mouse	(*460*)
	0.38	IV	Rat	(*406*)
Fe^{3+}	0.42	IP	Mouse	(*454*)

[a] Chelate.
[b] Insoluble hydroxide or phosphate.
[c] Various salts.
[d] Minimum lethal dose.

present in the lysosomes and, therefore, may not damage the cells.

Behavior in Living Organisms. A schematic model has occasionally been used to describe the behavior of Fe^{3+} and other transition element ions in different cells and in extracellular fluids (*447*). Due to the chemical similarities between Al^{3+} and Fe^{3+} this model could also be used for Al^{3+}. According to the model, the ions are believed to exist in four different forms (see Fig. 3): as free ions, as low molecular weight complexes, as reversible macromolecular complexes, or as irreversible macromolecular complexes.

"Free ions," i.e., $Al(H_2O)_6^{3+}$, $Al(OH)(H_2O)_5^{2+}$, $Al(OH)_2(H_2O)_4^{+}$, $Al(OH)_3(H_2O)_3$ and $Al(OH)_4^{-}$, occur in very low concentrations and are included mainly for theoretical reasons.

Low molecular weight complexes with organic acids, amino acids, nucleotides, phosphates, or carbohydrates,

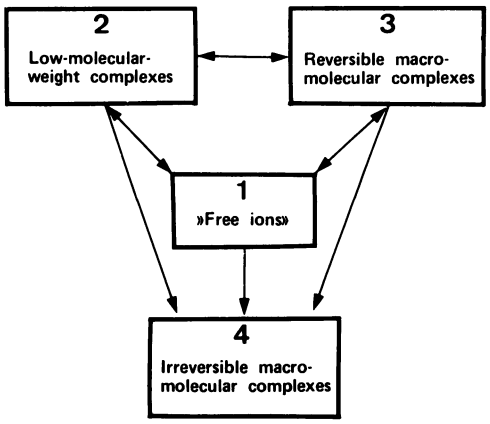

FIGURE 3. Schematic model of the intra- and extracellular behavior of Al^{3+} in organisms.

are often chelates and may be very stable. More than one chelate-forming ligand is often bound simultaneously, and these ternary or "mixed" complexes are probably common intermediates when Al^{3+} exchanges its ligands. The complexes are metabolically comparatively active, especially the nonpolar ones.

Al^{3+} has a very high affinity for a large number of proteins, polynucleotides, glycosaminoglycans, etc., and probably the main part of it may exist as reversible macromolecular complexes with these substances. Metabolically the complexes are much less active than the above small complexes and, thereby, contribute to the slowness of the Al^{3+} metabolism.

Certain bindings to macromolecular structures can be so stable that they are, practically speaking, irreversible—at least until the structure that bound the ion is itself destroyed. If Al^{3+} forms such complexes with structures that are themselves very stable, these should continue to accumulate Al^{3+} or might, sooner or later, be saturated with Al^{3+}. Considerable evidence indicates that the cell nucleus and the chromatin are the final destinations for Al^{3+} in the cells and that, consequently, any irreversible complexes should be found in these structures.

Previously, Al^{3+} was commonly used for producing leather by tanning hides, but it has now been replaced by Cr^{3+} and Zr^{4+} or by various organic compounds. This earlier use illustrates, however, an essential property of Al^{3+} in a biological environment, namely, to be able to form extremely stable complexes with polyanionic macromolecules (e.g., proteins, polynucleotides, glycosaminoglycans). In tanning, Al^{3+} is bound to anionic groups of amino acid residues in collagen (*367,462*). The ability of Al^{3+} to bind to polyanions, together with its tendency to polymerize with surrounding Al^{3+} ions by forming -OH- bridges (often wrongly called "olation") means that Al^{3+} has a considerable ability to crosslink polyanionic chains. If a single Al^{3+} ion is unable to bind two anionic groups, as the distance between them is too great, a suitable length polymer of Al^{3+} and OH^{-} can always be formed (*462*).

Al^{3+} and Cr^{3+} tannage also illustrates chemical coop-

erativity, whereby the binding of additional cations to collagen increases the stability of the former complexes. At a particular threshold value, the complexes become practically irreversible. Cr^{3+} tannage, that has been more closely studied than Al^{3+} tannage (*367*), binds about 4% chromium—expressed as Cr_2O_3 per dry weight of hide—equivalent to 2.7% Cr^{3+} (*463*). If the same molar amount of Al^{3+} is bound, then the weight ratio will be 1.4%. If the ratio cation/polyanion is below the threshold value, an irreversible binding could still be possible if the cations were able, by random diffusion, to form sufficiently large clusters in which the threshold value was exceeded and irreversible complexes could form. These clusters could then grow by binding to themselves even more cations and polyanions. The process would resemble the crystallization in a supersaturated solution after a crystallization nucleus has formed. Such a redistribution from a homogenous mixture of interacting cations and polyanions to clusters with a high concentration of cation would, however, probably proceed extremely slowly since it probably would take a long time before clusters of the critical size are formed and the irreversible binding sites in these then would have to compete with a far greater number of almost irreversible binding sites (clusters of subcritical size). *In vivo* the conditions for this hypothetical process ought to be most suitable in the cell nuclei, where polyanionic DNA is present at a very high concentration. Possibly, the process could also take place in collagen-rich connective tissues. However, to the knowledge of the author, there has been no report that this process actually has been observed in living tissues.

General Metabolism. A hypothetical model of Al^{3+} metabolism, based on the integrated information on Al^{3+} and the Al-like ions, is schematically illustrated in Figure 4 (the numbers in the figure refer to the different sections in the following discussion).

1. INTESTINAL MUCOSA: All Al^{3+}-like ions show very little intestinal absorption. It has been reported that $^{67}Ga^{3+}$ and $^{114m}In^{3+}$ are taken up by the intestinal mucosa in about the same proportion as $^{59}Fe^{3+}$ (*212*). Therefore, it is not unreasonable to assume that the mucosa also takes up a considerable fraction (perhaps 10–20%) of the Al^{3+} normally present in food; but Al^{3+} is firmly bound in the mucosal cells and it is returned to the intestinal lumen when the cells die.

The net intestinal absorption of Al^{3+} has only been estimated indirectly as the sum of the urinary excretion and the probable rate of accumulation in the tissues. The latter can be estimated to maximally 2 μg/day in adults; if it were higher the total body content of Al^{3+} would be greater than actually reported. Since the daily urinary excretion is at least 20 μg it seems warranted to conclude that roughly 0.1–0.5% of the normal Al^{3+} in food (< 20 mg) is absorbed, only to be subsequently rapidly excreted, by at least 90%, in the urine (see Fig. 4). This normal rapid and large excretion causes the concentration of Al^{3+} in the tissues to increase rapidly in renal failure.

With increasing Al^{3+} intake, e.g., as antacids, the fractional absorption is greatly reduced. The reason is, probably, that an increased concentration of Al^{3+} in the intestinal tract increases the probability of Al^{3+} existing as polymers or precipitated hydroxide. However, the presence of complex-forming substances in the intestinal contents can maintain the Al^{3+} as monomers and perhaps even facilitate the actual transfer into the mucosal cells.

As already stated, the normal Cr^{3+} concentrations in various organs, blood plasma, and urine are about 2 to 3% of the corresponding molar concentrations of Al^{3+}, but the reported molar content in food is only roughly 0.3% of the Al^{3+} content (*371–373*). Therefore, if these values are correct, the fractional absorption of Cr^{3+} is about 10 times that of Al^{3+}. A reasonable explanation for the difference could be that Al^{3+} and Cr^{3+} exist in different forms in the intestinal contents. A considerable fraction of both ions is possibly present in foods as stable chelates formed by plants. However, the Al^{3+} chelates should be more easily split than the ligand-field-stabilized Cr^{3+} chelates on their passage through the acidic environment in the stomach. The absorbed Cr^{3+} complexes could also persist in a more stable form in the mucosal cells so that Cr^{3+}, to a lesser extent than Al^{3+}, would be bound irreversibly.

2. LUNGS: The lungs continually receive Al^{3+}, mostly as particles of Al silicates and other poorly soluble compounds. Some of these particles can be assumed to be taken up by the alveolar macrophages by phagocytosis, then transported up via the respiratory system and finally swallowed (Fig. 4). The remainder is probably taken up by macrophages in the lung tissue and remain indefinitely in the lungs. As mentioned earlier, the lungs have a higher Al^{3+} concentration than all other organs and the concentration increases with age (*46,103,125*).

3. PLASMA, TRANSFERRIN-BOUND: In the blood, Al^{3+} and similar ions are believed to be present almost exclusively in the plasma, where they (with the exception of Be^{2+} and Zr^{4+}) are bound mainly to transferrin. In^{3+} is more stably bound than Ga^{3+} (*219*). Possibly, Al^{3+} is then even more weakly bound because it has the smallest ionic radius of the transferrin bound ions. However, that Al^{3+} can nevertheless compete with Ga^{3+} in its binding to transferrin is shown *in vitro* (*464*) and by animal experiments where preadministered Al^{3+} strongly affected the metabolism of $^{67}Ga^{3+}$ (*143*).

The literature gives many examples of how the ions in question after intravenous administration are metabolized differently due to the mode of administration. Simple salts, dissolved at high or low pH, easily form colloids when mixed with plasma, while stronger complexes generally bind to transferrin. Administration of large quantities of ions also increases the risk of colloids forming. These sources of error should, to a greater extent than previously, be recognized in *in vitro* experiments and metabolic studies with Al^{3+}-like ions. Consideration of this is also necessary when interpreting older literature.

Plasma elimination of IV-administered radionuclides of different Al^{3+} like ions have varied considerably, mostly due, it appears, to the above differences in the mode of administration. Cr^{3+} seems to have shown the slowest elimination from plasma. Half the intravenously admin-

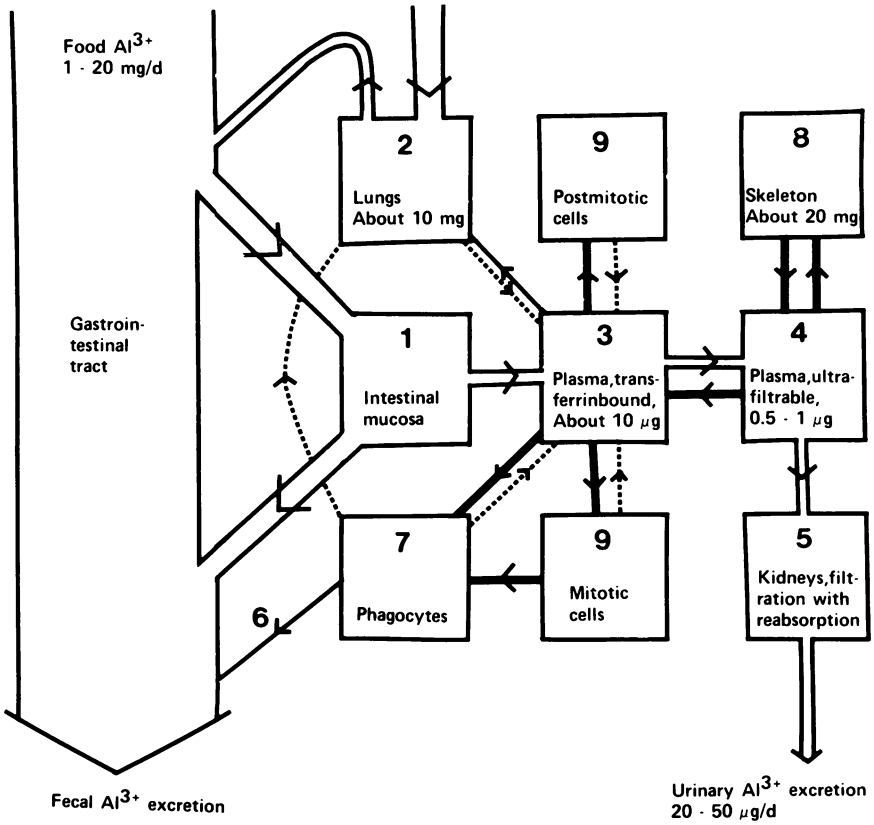

FIGURE 4. Hypothetical model of Al^{3+} metabolism in humans. The numbers refer to the corresponding text.

istered dose of $^{51}Cr^{3+}$ in rats is reported to have been eliminated from the plasma within 3 to 6 hr (*401–403*), while half of the administered Be^{2+} probably was eliminated from the blood in less than 1 hr (*322,465*), and according to one report in 3 hr (*309*).

If an adult person is normally assumed to excrete 30 μg Al^{3+}/day (Fig. 4) and is assumed to have a plasma Al^{3+} concentration of 3 μg/L (see Table 3), then the renal clearance can be estimated to about 7 mL/min (or even less if the plasma concentration were higher). This corresponds to about 5% of the normal glomerular filtration rate. If at least 90% of the absorbed Al^{3+} is excreted via the kidneys it can also be considered to represent the total plasma clearance. The normal half-life for plasma Al^{3+} would then, with a 2.5 L plasma volume, be roughly 4 hr. This is in good agreement with a determination in dogs of 4.6 hr (*466*), and with the above mentioned reports for Cr^{3+} elimination in rats.

4. PLASMA, ULTRAFILTRABLE: Within a concentration range of 25 to 200 μg/L, 10 to 30% of Al^{3+} in human plasma is reported to be ultrafiltrable (*45,54,55*) with a tendency towards a lower ultrafiltrable fraction with a lower total concentration (*55*). After IV administration of Al^{3+} to dogs that resulted in a maximal plasma concentration of 6000 μg/L, a renal clearance was determined corresponding to half the glomerular filtration rate of the animals (*52*). Taken together with the above estimations of the renal clearance at lower Al^{3+} levels, this could imply that at the normal level of Al^{3+} only about 5% is ultrafiltrable, which compares closely to the directly recorded values for Ga^{3+} (*218*, In^{3+} (*259*), Sc^{3+} (*279,467*), and Cr^{3+} (*392,468*). The findings also indicate that the ultrafiltrable fraction greatly increases with higher concentrations, whereby the renal clearance also increases. The same concentration dependency has been demonstrated for the ultrafiltrable fraction and the renal clearance of Cr^{3+} (*469*). Nevertheless, the normal ultrafiltrable fraction of Al^{3+} might be greater than the stated 5% if a tubular reabsorption existed, which would tend to reduce the clearance values obtained (see below and note 7).

If the ultrafiltrable fraction is supposed to be 5%, this would imply that in the extracellular fluid Al^{3+} normally is the predominant ultrafiltrable cation with a charge higher than +2. The corresponding molar concentration of Cr^{3+} is about 30 times lower and the concentration of Fe^{3+} (*447*) roughly 1000 times lower.

5. KIDNEYS: That a certain renal tubular reabsorption of Al^{3+} probably exists is indicated by the intravenous administration of a large dose of Al^{3+} to rats that resulted in an accumulation of Al^{3+} in the cells of the proximal tubules (*133*). Intravenous administration of large doses of Ga^{3+} to rats and humans also damaged the proximal tubules and this effect could partly be avoided by increased urinary volumes (*213,253*). In high doses equivalent to LD_{50}, In^{3+} (*260*) and Be^{2+} (*457*) also produce such kidney damage, and in idiopathic hemochromatosis hemosiderin (Fe^{3+}) is accumulated in the proximal kidney tubules (*470*). Different Al^{3+} and Cr^{3+} complexes are, however, reabsorbed to a varying extent

(Cr^{3+}-EDTA, for example, is not reabsorbated at all and is therefore used for measuring the glomerular filtration rate). It is most likely that the total extent of reabsorption of Al^{3+} and Cr^{3+} is also quite variable.

Presumably, reabsorbed Al^{3+} should have a tendency to be bound in the tubule cells. After intravascular hemolysis, hemosiderin accumulates in the tubule cells if haptoglobin is not able to bind all the free hemoglobin, which is therefore excreted in the kidneys and reabsorbed by the tubule cells. Generally, this hemosiderin is regarded not to return to the body pool of Fe^{3+} but is eliminated as the tubule cells are detached and replaced. This is probably what would also occur with Al^{3+}. It has, moreover, been proposed that Al^{3+} could be eliminated by exocytosis from the cells to the tubular lumen (*133*). Any possible reabsorption of Al^{3+} in the renal tubules could then be regarded only as a delayed urinary excretion, which does not—in long-term studies—reduce the total quantity excreted.

6. FECAL EXCRETION: After intravenous administration of $^{67}Ga^{3+}$ (*210,212,217*), $^{114m}In^{3+}$ (*212,260*), $^{46}Sc^{3+}$ (*285*), $^{51}Cr^{3+}$ (*366*), and $^{7}Be^{2+}$ (*311,319,320,471*), excretion via the gastrointestinal tract has been demonstrated, and such an excretion has also been proposed for Al^{3+} (*52,472*). The best investigated Al^{3+}-like ion is Ga^{3+}, which, in tests performed with rats, showed that main part of the gastrointestinal excretion occurs via the mucous membrane of the small intestine and that bile is responsible for at most 20% (*210,217*). The excretion has only been monitored during the first 1 to 3 days and, during this time, somewhat more than 10% of the given dose has been recovered. Also In^{3+}, Cr^{3+}, and Be^{2+} have only been investigated in short-term studies, often with rats. For Ga^{3+}, the fecal excretion was reported to be roughly 1/3 of the urinary excretion, for In^{3+}, 1/3–1/4 (*212,260*); for Cr^{3+}, 1/3–1/4 (*366*) or 1/2–1/3 (*402*); and for Be^{2+}, 1/3–1/4 (*311,319,471*) or, according to a few other reports, about 1/10 (*319,320*). The excretion of Sc^{3+} has been followed in longer studies performed in humans (*285*). During the first 24 hr after IV administration of $^{46}Sc^{3+}$–NTA, about 2% was excreted in the urine. The excretion rapidly declined so that only about 3% was excreted in the urine after 15 days. Fecal excretion during the first days was very low but then increased up towards 1% daily, and thereafter slowly declined. After 15 days, 7.5% had been excreted totally (see note 8).

It appears likely that fecal excretion of Al^{3+} also occurs and that it amounts to roughly one-third of the urinary excretion, i.e., about 10 μg/day for adults. The above-cited investigations using various radionuclides were performed, however, during relatively short time spans and therefore generally represent the excretion of only the first half of the given dose. Some evidence exists that the metabolic route leading to elimination via the gastrointestinal tract is much slower than the route that leads to elimination via the kidneys (*215,260*). Therefore, it cannot be excluded that the final total fecal excretion for the Al^{3+}-like ions is of the same magnitude as the urinary excretion. This should probably also apply to Al^{3+} and, in this case, the intestinal absorption must be assumed to be correspondingly greater than previously stated. On the other hand, it cannot be excluded that the demonstrated fecal excretion of the various Al^{3+}-like ions is an effect of colloid formation occurring during the IV administration of the radionuclide. As discussed above, such colloids are easily formed and In^{3+} colloid, in contrast to soluble In^{3+}, has been shown to be eliminated mainly via the feces (*260*).

7. PHAGOCYTES: Nothing is known of the mechanism for intestinal excretion of the Al-like ions. In Figure 4 it is tentatively proposed that granulocytes and macrophages might be responsible. These have a well-documented high affinity for Ga^{3+} and should probably, as a result of phagocytosis of other cells, also accumulate Al^{3+}. Furthermore, they also have a high turnover rate and some are believed to migrate to the intestinal mucosa or lumen where they die. If the daily turnover of granulocytes is accepted to be 50 mL and the Al^{3+} concentration in dying granulocytes is supposed to be 2 mg/L (about fourfold higher than the average values for soft tissues), then it should suffice if only 10% of the granulocytes migrated to the intestines to give rise to an excretion of 10 μg/day.

8. CONNECTIVE TISSUE AND SKELETON: Al^{3+} and all other Al^{3+}-like ions discussed here, with the exception of Fe^{3+}, are bound to a considerable extent to the skeleton. This is particularly apparent if the ions are administered orally or as trace doses IV to avoid colloid formation. As can be expected, binding occurs chiefly in the metabolically active areas in the bone tissue (*237,296,297*).

Some evidence exists that Al^{3+} and the other Al^{3+}-like ions are bound more generally in connective tissues; the organ distribution of In^{3+}, for example, was considered to support this (*255,256*). The ions have very high affinity both to collagen (compare the use in tannage) and glycosaminoglycans. Organs especially rich in connective tissue, as skin and omentum, had remarkably high levels of Al^{3+} and Cr^{3+} with a Cr/Al ratio being in complete agreement with other organs, i.e., about 3% by molarity (see Fig. 2 and note 9). The ratio in the human environment is considerably lower (0.06% by molarity in the earth's crust) but Cr(VI) is taken up by the skin much more easily than Al^{3+}, and chromate in the environment could incidentally give the same ratio in the skin as generally in the body. Hair and nails also showed the same ratio. Thus, that the high Al^{3+} and Cr^{3+} levels in the skin originate from external sources cannot completely be excluded. However, Ga^{3+}, and In^{3+} when administered intravenously, are also bound in the skin in relatively high quantities (*214,237,263*), which suggests the existence of some form of internal transport to the skin.

Collagen, e.g., in the skin, should theoretically offer a suitable environment for local accumulation of Al^{3+}. Collagen has a very slow turnover rate and shows definite aging, the nature of which is still essentially unknown (*473*), but is believed to be caused by an increasing number of crosslinkages between the molecules. This aging results in a decreased ability of the protein to be de-

graded by collagenase (*474*). It is theoretically possible that Al^{3+} could contribute to this aging as it can well crosslink collagen and probably also can stabilize it against the enzymatic degradation (compare the difference in this respect between catgut and chromicized catgut). However, the amount of Al^{3+} found in the skin is so small—about 2 mg/kg wet weight (*21*), equivalent to about 0.03% molar fraction of the amount of Cr^{3+} bound in tanning (*367*)—that, at first glance, it appears improbable that it would be able to influence in any way the properties of the skin. After tannage, it is believed that only about 10% of the bound ions actually take part in the crosslinks (*367*). Therefore, the normal quantity of Al^{3+} could constitute instead 0.3% of the minimal amount needed for tanning. With extreme Al^{3+} loads (e.g., after chronic hemodialysis), 100 times higher Al^{3+} concentrations have been detected in many tissues. If the concentration increases just as much in the skin, which, so far as is known, has not yet been investigated, the concentration would possibly be sufficient to affect the properties of the skin but the effect might need ample time to develop.

If structures rich in collagen accumulate Al^{3+}, then this could possibly also explain the accumulation of Al^{3+} reported in the walls of the small blood vessels (at least in the brain) which has been detected with low-resolution electron microscopy with X-ray spectrometry (*139,475–478*) or directly by dissection and chemical analysis (*108*). The basement membranes that normally have a very slow turnover may be responsible for this accumulation which then might help to stabilize them to normal degradation. With new collagen continually being formed, the process would hypothetically lead to the thickening of the vascular basement membranes which characterizes aging (*479–481*). However, reverse causal relations are also possible; a thickening of the basement membrane means that the number of strong binding sites increases and, therefore, the amount of Al^{3+} in the basement membranes could passively increase.

9. CELLS: In most organs, Al^{3+} and the Al^{3+}-like ions are probably mainly bound intracellularly. The close correlation found between Al^{3+} and Cr^{3+} in different tissues, therefore, implies that these ions, both regarding cell uptake and elimination from the cells, are treated in the same fashion. The cell uptake of Fe^{3+}, Ga^{3+}, In^{3+} and Sc^{3+} probably occurs only from the transferrin bound fraction and the density of transferrin receptors in different organs presumably helps govern the organ distribution. Taking into account the many other metabolic similarities shown by the Al^{3+}-like ions, it is highly probable that Al^{3+} and Cr^{3+} are also taken up in this way.

It is well established that transferrin receptors on the cell bind the various transferrin-metal ion complexes to the cell surface as a first step, but the following steps have been studied only for Fe^{3+}. In erythropoietic cells there is possibly a special mechanism for Fe^{3+} uptake, which is not discussed here. For most other cells it is believed that the Fe-transferrin complexes enter the cells by pinocytosis and that the microvesicles formed fuse with lysosomes; due to the low pH, Fe^{3+} is rapidly liberated, and transferrin is presumably degraded. So far it seems probable that Al^{3+} and the other ions follow the same route, since Al^{3+} (*109,133,137,138*), Ga^{3+} (*245,246*), and In^{3+} (*261*) have in short-term experiments been found in considerable amounts in the lysosomes.

Large amounts of Fe^{3+} can be stored in the lysosomes of macrophages and many other cells and it is highly probable that similar ions can also be stored. For Fe^{3+} the storage form is mainly crystalline FeO(OH). For Al^{3+} it has been suggested to be microcrystalline $AlPO_4$ (*109,133,136,137*), Fe^{3+} can also readily be released from the lysosomes and transferred to the cytosol. The exact mechanism for this transfer is unknown, but since virtually all other cations need specific transport proteins to pass lipid membranes it is highly probable that some specific mechanism exists. Whether this hypothetical mechanism also transfers Al^{3+} to the cytosol is, of course, quite impossible to tell. However, biologically it would be somewhat surprising if it normally did, as Al^{3+} has no function in the cell and is potentially a danger to many cell functions. It seems more reasonable to assume that permanent storage of the Al^{3+}-like ions in the lysosomes would be a main strategy for cells to avoid such a danger. Such permanent storage would also correspond with the accumulation of Al^{3+} in the cell vacuole in plant cells. Part of this stored Al^{3+} could, possibly, later be expelled from the cells by some form of exocytosis, although no direct evidence is available.

If this were the entire intracellular metabolism of Al^{3+}, its ability to affect the cells would probably be very limited. However, there is a good deal of evidence that small amounts of Al^{3+}, indeed, reach the cytosol, this being the only way to reach the nuclear chromatin, in which it is clearly accumulated. This uptake to the cytosol—whether by some form of leakage from lysosomes or lysosomal residual bodies or by some special cell membrane process—is probably much slower than the already slow uptake by means of transferrin; but it is probably of much greater interest. Fe^{3+} taken up in the cytosol is mainly bound to ferritin, and even Ga^{3+} and In^{3+} are possibly bound in small quantities (*249,250,261*) but apparently no one has investigated if Al^{3+} is also bound. If any such binding occurs, it is presumably of little importance quantitatively for the intracellular metabolism, otherwise Al^{3+} would probably not be accumulated in the chromatin. How Al^{3+} is then bound in the cytosol is unknown, but there are indications that Cr^{3+}, taken up in the cytosol as Cr(VI) and reduced to Cr^{3+}, mostly is in the form of comparatively low molecular weight complexes (*407*). Such complexes could easily enter the nucleus and be bound in the chromatin if this would produce the most stable complexes.

Ga^{3+} and In^{3+} taken up by cells in cell labeling procedures with 8-hydroxyquinoline or acetylacetone should also be located in the cytosol. After labeling with Ga^{3+}, In^{3+}, or Cr^{3+} there is very little leakage of the label from the cells; in fact, no investigation seems to have shown any elimination whatsoever from intact cells. Therefore, there might not be any way for cells to eliminate Al^{3+}-like ions from the cytosol–nucleoplasm compartment.

Tissue occurrence cannot be dependent on the uptake mechanisms only. Al^{3+} bound in cells that are continually being replaced must presumably be eliminated from the tissues at the same rate as the cells are eliminated and it is then, probably, transferred to the phagocytes that are responsible for the final cell elimination. Long-lived postmitotic cells could, on the other hand, continue to accumulate Al^{3+}. From the point of view of the general metabolism it can be suitable, therefore, to discriminate between mitotic cells and postmitotic cells (see Fig. 4). However, from the point of view of possible cell effects, it is probably much more important to discriminate between different intracellular compartments.

Special Organ Metabolism

ERYTHROCYTES: It is generally held that blood Al^{3+} is mainly found in the plasma and that the concentration of Al^{3+} in erythrocytes is, consequently, lower than the plasma concentration. This view originates, however, from the time when the normal plasma concentration of Al^{3+} was believed to be 100-fold greater than the present day concepts. This belief has most probably also been strengthened by observations made after artificial administration of Al^{3+}, e.g., through hemodialysis. In fact, no recent, well-documented investigation regarding the concentration of Al^{3+} in erythrocytes appears to have been published, but one investigation has stated that the concentration in whole blood is only 1.6 ± 1.3 μg/L (*482*). Since the normal Al^{3+} concentration in almost all other tissues in the body is at least 100 times that of the plasma concentration, it would be rather remarkable if the concentration in erythrocytes was lower than in plasma (especially as immature erythrocytes probably bind considerable amounts of Al^{3+} to the cell membrane and, possibly, may take up some part of it). The lack of nucleus and lysosomes in the mature erythrocytes could explain their low Al^{3+} concentration in comparison with other cells.

GRANULOCYTES AND MACROPHAGES: No known investigation has been performed regarding the uptake and metabolism of Al^{3+} and the Al^{3+}-like ions in granulocytes, with the exception of Ga^{3+}, which is readily taken up. Considering the other similarities between the metabolisms of the ions it seems probable that granulocytes also take up Al^{3+}.

If Al^{3+}-like ions are administered *in vivo* in such large quantities that colloids are formed, these are almost solely taken up by the macrophages of the reticuloendothelial system. *In vitro*, alveolar macrophages could only take up colloidal Be^{2+} phosphate and not soluble Be^{2+} complexes (*483*). Therefore, the direct uptake of Al^{3+} from plasma to the macrophages is possibly also small. If Be^{2+} is administered in large quantities, then the cells first taking it up are killed and a redistribution, particularly to the macrophages in the spleen, of Be^{2+} is observed after a few days (*484*). Also Sc^{3+} (*283,285*) and Ga^{3+} (*214*) showed a secondary increase in the spleen, which could indicate a redistribution. Al^{3+} from dead cells should, consequently, also be transferred to the macrophages. Any Al^{3+} that accumulates in mitotic cells (with the exception of epithelial cells which are expelled from the body), could then, sooner or later, be found in the macrophages; which could serve as the main final destination for the Al^{3+} metabolism.

LYMPHOCYTES: The uptake and contents of Al^{3+} in lymphocytes has apparently not been studied, and only one investigation has been found regarding the Al^{3+} concentration in normal human lymph nodes (*22*). According to this investigation, the concentrations of Al^{3+} and several of the Al^{3+}-like ions were very high; even higher than the corresponding concentrations in the lungs. No information is given regarding the origin of the lymph nodes but possibly they were lung hilus nodes (*485*). Fairly high lymph node uptakes have been observed after IV administration of Ga^{3+} (*214,237,243*), In^{3+} (*260*), Sc^{3+} (*281*), and Cr^{3+} (*376*) but it has not been investigated which particular cells take up the ions. That the lymphocytes are possibly responsible, however, is indicated by the high affinity of Ga^{3+} towards most lymphoid tumors, deriving from both B- and T-cells.

Investigations concerning the uptake of Ga^{3+} in cultured lymphocytes (*486–488*) have so far given somewhat varying results but can probably be interpreted as follows. Lymphoblasts and myeloma cells readily absorb Ga^{3+} if transferrin is available, but the uptake by normal lymphocytes from peripheral blood is considerably less. There are also indications that part of the bound Ga^{3+} is only bound to the cell surface and can, therefore, be removed with trypsin without any serious damage to the cells (*488*). Even if circulating lymphocytes, consisting mainly of dormant T-cells, take up Ga^{3+} only slowly, this does not necessarily imply that lymphocytes *in vivo* cannot accumulate Al^{3+} as some lymphocytes can survive for several years and have a very condensed chromatin (see discussion below).

After acute severe poisoning with In^{3+}, a pronounced lymphopenia was observed in the lymph nodes, spleen, and thymus (*260*) and acute severe Be^{2+} poisoning induced a large decrease in the number of circulating lymphocytes (but an increase in the number of granulocytes) (*457*). When Be^{2+} was administered as a soluble complex with sulfosalicylic acid, a strong toxic effect was noted on the T-lymphocytes in the spleen, thymus and lymph nodes while, at the same time, B-lymphocytes in the bone marrow were almost unaffected (*327*). Be^{2+}-exposed individuals, with or without signs of berylliosis, have also repeatedly been reported to have a reduced tuberculin reactivity. Similarly, Ga^{3+} is reported to inhibit the cellular immunity (*256*). Therefore, Al^{3+} and the other Al^{3+}-like ions could, in some way, be particularly toxic for T-lymphocytes.

Intraperitoneal injections of $Al(OH)_3$ in mice resulted in a very strong reduction of the mitogenic effect of phytohemagglutinin (PHA) on T-lymphocytes and *E. coli*-polysaccharide (LPS) on B-lymphocytes (*489*). The effect remained unchanged for several weeks. Spleen cell suspensions from treated mice also inhibited the mitogenic effect on cells from untreated mice. The authors interpreted this as a sign of special suppressor cells being

formed. Another possibility is that Al^{3+} had accumulated in the spleen and had seriously damaged many cells which disintegrated later in the experiment. Liberated Al^{3+} could then influence the untreated cells. A similar inhibition of blast transformation has been observed with Cr^{3+} (*490*) and Be^{2+} (*491–493*). Nothing is known of the specific mechanism, but it is interesting to note that colchicine also inhibits the PHA-induced blast transformation (*494–498*). As Al^{3+} and colchicine have similar effects in some other situations, it is perhaps possible that some of the effects Al^{3+} has on lymphocytes concern the function of the microtubules.

PLATELETS: No investigations are known to have been published regarding either the presence of Al^{3+} in platelets or any effects on them caused by Al^{3+}. Among other ions, In^{3+}, after IV administration of a sublethal colloidal dose, induced a powerful but transitory (about 1 week) reduction of the platelet count (*260*).

ADRENALS: The adrenals are reported to have a high normal concentration of Al^{3+} (*21,28,103,104*) and Cr^{3+} (*21*) (see Fig. 2) and also to take up large amounts of Ga^{3+} (*214,237*) and Sc^{3+} (*499*) after intravenous administration. The specific cells that bind the ions are not known. However, it is reasonable to assume that these cells are, in fact, the adrenal medullary cells which are known to be "chromaffin" i.e. bind or react with chromium in histological specimens. The basis for the chromaffin reaction is believed to be Cr(VI) that oxidizes and polymerizes the catecholamines in the secretory granules and stains them brown. No direct binding of the Cr^{3+} formed is usually thought to occur. The catecholamines are believed to be stored in the secretory granules in the form of an ATP and Mg^{2+} or Ca^{2+} complex. Therefore, there is a possibility that Al^{3+} and Cr^{3+} could replace Mg^{2+} or Ca^{2+}. Both have high affinities to ATP and to catecholamines. The latter property is used practically in methods to isolate catecholamines from urine, tissue extracts, etc. with Al^{3+} hydroxide gels. Al^{3+} has also been used in a histological technique to demonstrate catecholamines. The brain of an anesthetized experimental animal is perfused with an Al^{3+}-containing buffer, whereby the catecholamines are "locked in" in their granules—greatly improving the later visualization by fluorescent microscopy (*500*).

PARATHYROIDS: In the literature, the parathyroids have been associated with Al^{3+} in two different ways. According to one group, the human parathyroids contain about 3.25 mg Al^{3+}/kg wet weight, which is 5 to 10 times more than in most other soft tissue organs (*105*). If Al^{3+} hydroxide was given orally (1 g/day in humans), the parathyroid concentration rose linearly with the given dose to about 40 mg/kg. The same authors have also reported that normal parathyroids, and particularly parathyroid adenomas, after IV administration take up $^{67}Ga^{3+}$ and $^{46}Sc^{3+}$ in roughly 10 times greater quantities than the thyroid and 30–40 times greater than muscle (*241*). In the cell culture of bovine parathyroid cells, Al^{3+} inhibited the hormone secretion even more than Ca^{2+} (*501*), but the Al^{3+} concentration used was very high, 0.5 to 2.0 mmole/L. There is probably no definite indication that Al^{3+} *in vivo* affects the parathyroid function (*502,503*). In parathyroids from patients on chronic hemodialysis having secondary hyperparathyroidism, Al^{3+} was localized to the lysosomes, in the principal and the oxyphilic cells, together with an equivalent amount of phosphorus (*137*).

The other association between the parathyroids and Al^{3+} is in conjunction with the earlier mentioned reports that the parathyroid hormone might increase absorption of Al^{3+} in the intestines (*43,44*). These reports have, however, been questioned by other workers (*45–47*).

BRAIN: The concentration of Al^{3+} in the brain is quite easy to determine and many reports in the literature state the average normal concentration in human adults to be about 0.5 mg/kg wet weight (see Table 4). Just how rapidly or slowly Al^{3+} is taken up and metabolized by the brain has, however, been impossible to assess. With IV administration of different radionuclides of the Al^{3+}-like ions (mostly in experimental animals) the uptake is reported to be at most 10% of the uptake registered for several soft-tissue organs (see note 10). The brain uptake was also less than 10% of the value expected for the dose if it had been evenly distributed in the experimental animal (see note 11). The uptake of Ga^{3+} in the human brain is reported to be 0.1% of the given dose/kg (*214*), i.e., at most 0.15% in the entire brain compared to about 2% if the dose had been evenly distributed in the body.

Due to the great similarities otherwise found for the metabolism of Al^{3+} and the other Al^{3+}-like ions, there seems to be good reason to believe that Al^{3+} is also taken up more slowly by the brain than by other organs. By assuming the normal brain uptake fraction of Al^{3+} to be the same as for the other ions, i.e., about 0.15% of the dose entering the blood, and also assuming the normal intestinal uptake of Al^{3+} per day to be 50 μg (urinary excretion + probable intestinal excretion + maximal accumulation, see discussion above), then the normal daily uptake in the brain can be estimated to be 0.075 μg. It would take, therefore, 36.5 years to accumulate 1 mg, which is the total quantity found normally in the brains of older individuals (see note 12).

The uptake can also be assessed in another way. The mechanism governing passage of Al^{3+} across the blood-brain barrier and its concentration in the cerebrospinal fluid are completely unknown (see note 13). For assessment it is, nevertheless, assumed that the passage occurs entirely through passive diffusion with complete equilibration, whereby 5% of the Al^{3+} present in the plasma, as ultrafiltrable complexes, is transferred to the cerebrospinal fluid. If the normal Al^{3+} concentration in plasma is assumed to be 3 μg/L and the production of cerebrospinal fluid to be 0.5 L/day, then, again, 0.075 μg Al^{3+} would cross the barrier per day.

Naturally, these estimations are based on information and assumptions that are uncertain. As evidence to support the plausibility of the estimations, a comparison of the total brain content and the total body content of Al^{3+} in cases of dialysis related encephalopathy can be made. The brain content is about 4.5 mg (3 mg/kg wet weight,

Table 10. Concentrations of Al^{3+} in the brain associated with lethal poisoning, according to the literature.

Species	Mode of administration	Stated concentration, mg/kg dry weight[a]	Stated or probable concentration, mg/kg wet weight	References
Cat	Intracerebral injection	4–6	1–1.5	(*107*)
—	Intracerebral injection	7	1.75	(*106*)
Rat	Intracerebral injection	16	4	(*106*)
—	Intraperitonal injection		2.2	(*109*)
Rabbit	Subcutaneous injection	5–15	1.25–3.75	(*110*)
—	Intracerebral injection	15–35	3.75–8.75	(*111*)
Man	Inhalation		5	(*114*)
—	Hemodialysis	8.9 ± 4.3	2.2 ± 1.1	(*122*)
—[b]	Hemodialysis	5–20	1.25–5	(*123*)
—[b]	Hemodialysis	3.5–4.6	0.9–1.2	(*505*)
—[c,d]	Hemodialysis	26.5 (20–33)	6.6 (5–8)	(*119*)
—[c]	Hemodialysis		5.5 ± 1.6	(*118*)
—[c,e]	Hemodialysis	25 (8–50)	4.2 (1.3–8.3)	(*23,46*)
—[c]	Hemodialysis	12.4 ± 4.9	3.1 ± 2.2	(*113*)
—[c]	Hemodialysis	13	3.25	(*461*)
—[f]	Hemodialysis	36–142	9–35	(*102*)
—[b,c]	Oral antacids	80	20	(*506*)

[a] In cases where the concentration is given only as dry weight the value has been divided by 4 to get a probable wet weight concentration (*21*). The concentration per fat-free dry weight has, in a similar manner, been divided by 6.
[b] Lacks own reference value.
[c] Refers to gray matter.
[d] Reports high normal value 9.5 (4–14) mg/kg dry weight.
[e] Reports fat-free dry weight.
[f] Reports high normal value 6.1–17.8 mg/kg dry weight.

see Table 10) and the total body content roughly 3 g (*23,461*). The total body content in renal failure can probably be assumed to be equal to the amount unintentionally administered in the blood. With this mode of administration, therefore, the uptake in the brain can be estimated to approximately 0.15%, i.e., the same percentage as used in the above estimations. However, it would be surprising if the absence of urinary excretion did not increase the uptake in the brain. If this implies that the normal uptake in the brain is even lower than 0.15%, or if some of the Al^{3+} taken up by the brain later leaves it, is, however, uncertain.

If the other basis for assumption is used, it could roughly be assumed that the "extra Al^{3+} content" in the brain, about 4 mg, was taken up during a period of 2 years [average time on dialysis (*504*)] and that the average concentration in the plasma, during this time, was 200 μg/L (*56*). The uptake in the brain would then be about 5.5 μg/day and if this quantity passed the blood–brain barrier together with 0.5 L cerebrospinal fluid, the passage fraction would be 5.5% of the plasma concentration, i.e., almost identical to the amount accepted in the previous estimations. However, at this blood level the ultrafilterable fraction should, according to the discussion above, have been about 20%. The reason for the discrepancy is obscure.

Because Al^{3+} is neurotoxic and presumably lacks any form of function in the brain, the concentration encountered in lethal poisoning is of just as much interest as the normal concentration. In Table 10, the available information from the literature is given. There is considerable agreement that severe toxic effects occur at a concentration of 1.5 to 5 mg/kg wet weight. The occasional higher values can partly be explained by calibration differences in the methods of analysis used. Notably, the toxic level seems to be fairly independent of the mode of administration. The stated level implies that lethal poisoning can occur at a concentration that is only 3 to 10 times higher than the average normal concentration in adults (0.5 mg/kg wet weight, see Table 4) or only 2 to 7 times higher than the concentration found in older individuals. A similar relationship was obtained with investigations on isolated heterochromatin from neurons. With lethal Al^{3+} poisoning, the concentration was increased 5.4 times in cats and 3.9 times in rats compared to the normal concentrations found in heterochromatin (see discussion below) (*106*).

In summing up, it can be noted that both the normal and toxic concentrations of Al^{3+} in the brain are documented with more than 15 articles that are in nearly complete agreement and which imply that only a rather narrow margin exists between normal and lethally toxic levels. This fact has, however, seldom been observed or discussed in the literature (*507*).

MILK: The occurrence of Al^{3+} in milk has mostly been discussed from the viewpoint of milk being a foodstuff in which the Al^{3+} concentration has been studied. As a result, the determinations have generally been performed on cow's milk in the form that it reaches the consumers. The possibility exists, therefore, that Al^{3+} might have been added and that the values do not solely represent the biological secretion. The stated values are given in Table 11. Several of the values were determined at the time the concentration of Al^{3+} in plasma was generally regarded to be almost 100-fold higher than the values later found to be correct. Therefore, a certain suspicion exists that the concentration in milk is also overestimated. One investigation (*508*), performed on

Table 11. Normal concentrations of Al^{3+} and Cr^{3+} in milk according to the literature.

Species	Al^{3+}, μg/L	Cr^{3+}, μg/L	Year	Reference
Cow	460 (150–970)		1954	(*508*)
—	470 ± 235[a]		1961	(*509*)
—	700 ± 930		1959	(*510*)
—	1770 ± 243		1964	(*511*)
—	2000		1970	(*16*)
—	1100		1979	(*513*)
—	400–1000		1980	(*514*)
—	900[b]		1982	(*452*)
Rabbit	1500		1984	(*515*)
Human	330 ± 42		1964	(*511*)
—	350 ± 160		1979	(*513*)
Cow		13 ± 12	1959	(*510*)
—		8	1971	(*516*)
—		10–20	1980	(*514*)
Human		43–80	1968	(*517*)
—		11.6 (6.4–18.5)	1971	(*516*)
—		1	1980	(*387*)
—		0.3 (0.06–1.56)	1984	(*518*)

[a]d Recalculated assuming milk gave 0.64% ash (*511*).
[b] Analyzed, 1969.

fresh milk from individual cows, reported concentrations that varied considerably from animal to animal despite identical fodder intake. Month to month variations were also observed and were believed to be due to differences in the Al^{3+} content of the various fodder.

If the lowest values in Table 11 are true and the Al^{3+} concentration in milk is in the range of 300 μg/L, then lactation must be an important factor that should influence the Al^{3+} metabolism considerably. If 200 μg is secreted daily in humans, then, for example, the total skeletal content of Al^{3+} would be exhausted in 100 days, on the condition that absorption and excretion via other routes remain unchanged.

Reported values for Cr^{3+} concentration in milk from cows and humans are also given in Table 11. Any certain conclusion regarding the true concentration is difficult to draw. Assuming it to be 15 μg/L, then the concentration would be 2.6%, by molarity, of the above discussed 300 μg/L Al^{3+} concentration. However, the latest values are substantially lower.

Investigations with $^{67}Ga^{3+}$ suggest that the secretion of Al^{3+} and Cr^{3+} could indeed be large. Two cases have been published (*239*,*519*), where Ga^{3+} scanning was performed in women during lactation and the concentration of Ga^{3+} was measured in the milk. Both investigations showed a powerful breast uptake of the radionuclide. In the first investigation, the secretion was monitored for 8 days (*239*). After correction for physical decay, the Ga^{3+} content in the milk decreased almost exponentially to half the initial value in 9 days. The other investigation only reported values for the 4th and 5th days after administration (*519*). After these values were also corrected for physical decay, both investigations are found to be in complete agreement. Both also lack information on the quantity of milk produced. If it is assumed to have been 0.75 L/day, then it can be estimated from the reported data that no less than about two-thirds of any administered trace dose of stable Ga^{3+} should be secreted in the milk during a period of about 2 weeks. As a comparison, only about 1.3% of a trace-dose of $^{7}Be^{2+}$, intravenously administered to a cow, was secreted in the milk during 5 days (*312*). It is thus possible that the binding to transferrin or lactoferrin is, in some way, necessary for the high secretion in milk. It is also of interest to note that milk tends to have a very low Fe^{3+}/Al^{3+} ratio (see Table 6).

PLACENTA: No detailed investigation of how the placenta can metabolize Al^{3+} appears to have been performed. However, the placenta has been shown to take up some of the Al^{3+}-like ions very actively. For example, primarily $^{114m}In^{3+}$ but also $^{67}Ga^{3+}$ and $^{51}Cr^{3+}$, have been clinically used in localizing the placenta.

In experiments with pregnant rats during the latter part of gestation, roughly 40% of the administered dose of $^{113m}In^{3+}$ was bound in the placentas within 5 hr (*266*). A simultaneous trial with ^{125}I-labeled transferrin was considered to indicate that In^{3+} was not merely bound, as In^{3+}-transferrin, to the transferrin receptor on the cell surface. It was assumed, therefore, that the placental cells had indeed taken up the radionuclide. However, there was a very poor transport of the radionuclide over the placentas to the fetuses compared to $^{59}Fe^{3+}$ administered simultaneously.

When pregnant rabbits received a mixture of $^{113m}In^{3+}$, $^{67}Ga^{3+}$, $^{51}Cr^{3+}$, and $^{59}Fe^{3+}$ intravenously, the placentas accumulated proportionally 5 times more In^{3+} and 2.5 times more Ga^{3+} than Fe^{3+} but only 3 times less Cr^{3+} compared to Fe^{3+} (*520*). Fe^{3+} was transferred across the placenta to a 150 to 400 times greater extent than the other ions. The placenta appears, therefore, to bind the ions very effectively and thereby, presumably, protects the fetus. The ratio placenta/fetus for In^{3+} was no less than 853, calculated per gram tissue.

In pregnant rabbits, the placental uptake of $^{67}Ga^{3+}$ was 10 to 20 times higher per gram tissue than the uptake in the liver or spleen and 100 to 1000 times higher than in the fetal kidney or liver (*521*).

However, minute amounts are evidently transported over the placenta. $^{67}Ga^{3+}$, taken up by the mouse fetus has been shown by autoradiography to be localized mainly in the skeleton (*522*). Investigations on pregnant hamsters that received an intravenous dose of In^{3+} and Ga^{3+} equivalent to about one-tenth of LD_{50}, produced 12% stillborns and malformations in 38% of the living fetuses (*268*). The most common malformations were found in the skeleton, often the toes. Transferrin is necessary for transport over the placenta as was shown by saturating transferrin with Fe^{3+} which then blocked the teratogenic effects (*269*). Similar tests have also been carried out using Al^{3+} (*523*). If Al^{3+} was administered to pregnant rats intraperitoneally in a dose equivalent to about 20% of LD_{50}, then no effects were detected in the fetuses. However, if the dose was increased, it resulted in an increased frequency of skeletal malformations if the dose was given in day 14 to 18 of gestation but not if it was administered earlier. The frequency of stillbirths also increased.

Biochemical and Cell Metabolic Effects

ENZYMES AND MEMBRANE TRANSPORT SYSTEMS: There is a paucity of studies investigating the interactions between enzyme systems and the Al^{3+}-like ions. Still it may be possible to distinguish a common pattern with enzymes acting on organic phosphate compounds being most sensitive. Particularly interesting is the tendency of Al^{3+} to form very stable complexes with ATP, which has made it necessary to purify commercial ATP preparations before they are used in enzyme kinetic experiments (*149*). Potentially this interaction could influence, directly or indirectly, an almost unlimited number of biochemical reactions in the cells. Another interaction, possibly of similar importance, is the recently reported effect on calmodulin.

However, the reported investigations have mostly been performed *in vitro*. Even if a purified enzyme is strongly inhibited *in vitro* by Al^{3+} levels existing in the cells, it does not justify the conclusion that the enzyme is inhibited also *in vivo*. On the other hand, enzyme reactions that are reported not to be inhibited *in vitro* could, nevertheless, be inhibited *in vivo*. Common to most of the investigations performed on Al^{3+} inhibition of enzymes has been that very short preincubations have been used to allow the ion to interact with the enzyme or its substrates. Instead, too high Al^{3+} concentrations have been employed that must have led to the formation of Al^{3+} colloids.

Among enzymes thus reported not to be inhibited *in vitro* are $Na^+ + K^+$–ATPase and Mg^{2+}–ATPase (*163*). The reported lack of inhibition is somewhat surprising, as Cr^{3+} slowly inhibits $Na^+ + K^+$–ATPase (*363*) and also inhibits Ca^{2+}–ATPase (*364*) and Mg^{2+}–ATPase (*365*). However, according to a recent report (*524*), Al^{3+} inhibited $Na^+ + K^+$–ATPase *in vivo* (fish gills). Similar results have also been reported for Cr^{3+} (*525*).

CELL NUCLEUS AND CHROMATIN: Al^{3+} has a strong affinity to chromatin and DNA and it is unquestionably to these structures that the ions are bound in the cell nucleus. Just how specifically Al^{3+} and the other ions are bound to DNA may also be illustrated by their histochemical applications. As already mentioned, In^{3+} has been proposed as a selective DNA staining agent for electron microscopy. The most widely used substance for histological staining of cell nuclei and chromatin, hematein (oxidized hematoxylin), requires a metal ion as a mordant. Al^{3+}, Cr^{3+}, and Fe^{3+} have mostly been used for this purpose. The mordant acts, in reality, by "staining" the specimen with metal ions and then hematein is bound as a chelate to the bound ions (*526*). Besides, hematein has also been used in a spectrophotometric method for Al^{3+} determinations (*527*). Using Al^{3+} as a mordant, and under suitable conditions, hematein can selectively stain chromatin in the cell nuclei in histological sections (*526*).

Cell nuclear accumulation takes place mostly in the heterochromatin. In one experiment with cats and rats, Al^{3+} was injected directly into the cerebrospinal fluid and the animals were subsequently killed. Neuron cell nuclei were isolated and sonicated and the chromatin was separated into euchromatin and heterochromatin by centrifugation. It was shown that 89 to 90% of the chromatin-bound Al^{3+} was recovered in the heterochromatin fraction, which comprised only 25% of the total chromatin content. Similar accumulation in the heterochromatin was found in neuron cell nuclei from deceased humans who had not suffered from any known neurological disease and from patients that died of Alzheimer's disease or dialysis-related encephalopathy (*106*).

Chemically, it is very reasonable to believe that the more dense DNA structure of the heterochromatin would offer more stable binding sites for Al^{3+}. Heterochromatin is a gel of DNA and protein with a concentration of DNA-P close to 1 mole/L. The molar ratio Al/DNA-P was 0.12 in the above rat experiments. The concentration of Al^{3+} in the heterochromatin should therefore have been in the range of 50 to 100 mmole/L. Describing it in another way, the Al/DNA ratio was 1.05% by weight. This can be compared with the estimated Al^{3+} concentration of 1.4% (dry weight) in tanned leather (see discussion above). Even if the histones should also be included in these calculations, the ratio in heterochromatin is still not too far from that of leather. The Al^{3+} was probably not evenly distributed in the heterochromatin, either. This means that part of the heterochromatin in these experiments could well be characterized as "DNA-leather." Heterochromatin from patients with Alzheimer's disease has, in the same manner, an Al^{3+} concentration of 0.37%, compared to 0.2% for healthy individuals.

The Al/DNA-P ratio of euchromatin in the above rats (*106*) was only 0.0015, i.e., almost 100 times lower than in the heterochromatin and 10^3 to 10^4 times lower than the ratios in which Al^{3+}, *in vitro*, overstabilized the DNA double-strand structure. Therefore, it appears improbable that Al^{3+}, even in severe poisoning, has any such direct effect on active chromatin. Hypothetically, RNA synthesis could be a process that transports Al^{3+} away from the euchromatin and the cell nucleus, as RNA probably binds Al^{3+} as strongly as DNA does, and more strongly than the monomeric nucleoside triphosphates taken up by the nucleus for the RNA synthesis.

The Al/DNA-P ratio of whole, normal, human neuron cell nuclei (not only the heterochromatin) was, according to the quoted investigation, 700 μg/g DNA—equivalent to an Al/DNA-P molar ratio of 0.008 (*106*). A simple calculation shows that this must be a high ratio. The human body is believed to contain at least 10^{14} cells, and each of these contains 6×10^{-12} g DNA. This implies that the total body content of DNA is at least 600 g. The total normal Al^{3+} content of the body is, according to the discussion above, about 40 mg—most of which is probably not contained in cell nuclei (insoluble Al^{3+} compounds in the lungs and Al^{3+} bound in the skeleton). If it is assumed, nevertheless, that half of it is bound to DNA, then the average Al/DNA ratio would be 33 μg/g or about 0.0004 mole Al^{3+}/mole DNA-P.

That neurons contain at least 20 (possibly up to 100) times more Al^{3+} in their chromatin than the theoretical average for other cells in the body can, of course, be due to their long life. In cell division, Al^{3+} that may have

accumulated in the chromatin is probably divided equally in the daughter cells. In organs with mitotic cells, cell death constantly occurs. The accumulated Al^{3+} of the dead cells is then most probably taken up by phagocytes and can be removed from the organ. However, in neurons, Al^{3+} could accumulate continuously through an entire lifetime. Another possible reason for the high Al/DNA ratio could be that neurons are very large cells, with a typical perikaryonic volume of 1 to 10 pL (the axonal volume can be many times greater). The gray matter in the brain in older individuals contains, according to all accessible information, on the average 1 mg Al^{3+}/L (see discussion above). If half of that amount is contained in the neuronal perikarya (*112*) and the density of neurons is assumed to be 20×10^9 L^{-1} (*528*), then each neuron would contain on the average 25×10^{-9} μg Al^{3+}. If all this could accumulate in the 6×10^{-12} g DNA of the chromatin, then the Al/DNA ratio would be 4166 μg/g, which is more than enough to produce "DNA-leather" of all the heterochromatin. This calculation is based on a mean perikaryon volume of 2.35 pL (*528*), which gave a total perikaryon volume of 47 mL/L of gray matter and a mean intraneuronal Al^{3+} concentration of 10.6 mg/L. If the largest neurons of the body which have a volume of > 60 pL (diameter of approximately 50 μm), e.g., Betz's giant pyramidal cells, Purkinje cells and anterior horn cells in the spinal cord, should have the same intracellular Al^{3+} concentration and if they were able to accumulate all that Al^{3+} in the chromatin, then the ratio would be 106,000 μg/g DNA, i.e., in the entire chromatin, eight times higher than in Al^{3+} tanned leather; more than 3000 times higher than the theoretical average Al/DNA ratio for the whole body.

Even if these calculations may seem very theoretical, they can nevertheless illustrate how the largest cells could be most liable to Al^{3+} accumulation in the cell nucleus, at least when no cell division occurs. At the same time, the calculations probably show that large cells either contain small quantities of Al^{3+} in comparison to their size or that Al^{3+} in large cells is not predominantly located in the cell nuclei. But finally, the calculations perhaps also show that the low tissue concentration of Al^{3+} is, nevertheless, not so low that it would be quantitatively impossible for Al^{3+} to interfere with the functions of the cell nucleus, at least in large, long-lived postmitotic cells. Among other cells of this type, oocytes with a volume of 100 to 200 pL can be mentioned. The theoretical assumption that large cells are most liable to damage by Al^{3+} is also in agreement with experimental observations. Al^{3+}-induced neurofibrillary degeneration was observed chiefly in spinal chord anterior horn cells, giant pyramidal cells, Purkinje cells, and other very large neurons (*107,110,192,200,529*).

If Al^{3+} binding in the cell nucleus predominantly occurs in the metabolically inactive heterochromatin, then this is presumably less harmful for the cell metabolism (see note 14) than if an equal amount had been bound to the active euchromatin. Various mechanisms could still, theoretically, lead to interference with the cell functions.

Al^{3+} might, for example, condense and inactivate part of the euchromatin. Interphase euchromatin is mainly composed of 10 nm chromatin fibers consisting of nucleosomes arranged like beads on a string. If divalent ions are added, especially Mg^{2+} and Ca^{2+}, a new fiber is formed with a diameter of 30 to 40 nm, probably by formation of a superhelix of the 10 nm fiber (*530–533*). This new fiber can then condense even more. Conversely, if strong metal ion chelators are added to heterochromatin, the structure is broken up to the previous 10 nm fibers (*534*). Mg^{2+} and possibly Ca^{2+} are therefore assumed to stabilize the supernucleosomal chromatin structure in the condensed chromatin (in cooperation with the histones) and it has also been suggested that an increase of the Mg^{2+} (*535*) or Ca^{2+} concentration (*536*) would be an important factor for condensing chromatin to chromosomes prior to cell division. If Mg^{2+} has this stabilizing and condensing effect, then it is very likely that Al^{3+} possesses it too, and, in addition, that Al^{3+}—with its higher affinity to DNA—also partially displaces Mg^{2+}. Random clusters of Al^{3+} ions might therefore produce local condensations. This would give rise to new binding sites for Al^{3+} and small islands of condensed chromatin could possibly be able to grow in the way discussed earlier. However, the condensed chromatin would at the same time increase its density and might consequently follow the normal heterochromatin in the chromatin fractionation. The difference in the Al^{3+} concentrations between the two fractions would thereby apparently remain unchanged, but the quantity of heterochromatin should increase somewhat and the cell metabolism would probably be affected.

Al^{3+} could make the heterochromatin so "sticky" that it could capture and inactivate essential parts of the euchromatin. Al^{3+} and several similar ions appeared to make the chromosomes sticky (*182*), as Be^{2+} also did (*182,537,538*), and even normally the heterochromatin shows several signs of stickiness. It has a tendency to aggregate in contact with the nuclear envelope. Small portions of heterochromatin seem to unite easily into larger blocks and from one heterochromatin block bridges of heterochromatin are also often observed to other blocks, to the nucleolus or to the nuclear envelope (*539*).

If large amounts of Al^{3+} accumulate on the surface of heterochromatin blocks, then Al^{3+} should be able to form bridges to other parts of the chromatin, or to the nuclear membrane lamina, that comes in contact with the heterochromatin. It could also reduce the repulsion exerted by any negative net charges present. Euchromatin fibers could then show a tendency to adhere to Al^{3+}-rich heterochromatin blocks, temporarily at least. The tendency for small heterochromatin portions to combine into larger blocks would also likely increase and the larger blocks could be stabilized by Al^{3+} and become more permanent. Small, but for the cell function, important euchromatin portions could happen to be caught between the heterochromatin blocks as they united and, thereby, be enclosed and inactivated. The affected cells could degenerate slowly or die more suddenly due to deranged nuclear functions.

Finally, an excessive accumulation of Al^{3+} could inter-

fere with the meiotic or mitotic mechanisms in cases where cell division would later take place in the affected cells. Replication of "DNA-leather" would presumably function with considerable difficulty. If it was accomplished, the Al^{3+} present in the nucleus could make the chromatides so sticky that nondisjunction or chromosome breakage might occur. The heterochromatin could also serve as a passive "sink" for Al^{3+} that, later in cell division, could become mobilized and could affect the mitotic spindle function and thereby cause nondisjunction (see below).

Against the background of all the potential abilities, Al^{3+} thus appears to have to affect DNA and cell functions, it is very obvious that this seems to occur seldom in organisms both in the natural environment and in various test situations. Taking into consideration the general occurrence of Al^{3+} and its metabolic slowness, however, there need not be any discrepancy between theory and reality. Al^{3+} metabolism seems to be so sluggish that a severe Al^{3+} accumulation in the cell nuclei of humans is probably not normally possible in most organs, and would take several decades to occur in the brain and other possibly affected organs. Furthermore, as all individuals are exposed to Al^{3+}, any effects of accumulation would probably be interpreted as normal changes or aging. Accumulation of Al^{3+} in certain cell nuclei might, therefore, even be one of the normal aging mechanisms. Such a hypothesis also fits neatly with the idea held by many authors (*540–545*) that progressive heterochromatization may be a main cause of normal aging. It also fits with the coarsened nuclear structure and increased staining of the chromatin that are often seen in old cells.

Al^{3+} has not shown any pronounced effects in mutagenicity tests and only a few reports seem to indicate that Al^{3+} can affect mitosis and meiosis, cause chromosome damage, or various types of chromosome mutations (*182–184*). However, this lack of observed effect could also be due to the sluggish metabolism; Al^{3+} could simply not have time enough to be taken up by the test organisms or to affect DNA. Also Cr^{3+} and Be^{2+} have shown negative results in most mutagenicity tests, despite many indications that they are mutagenic once inside the organism. Recently, it has also been pointed out that traditional mutagenicity tests and other short-term tests could have a general tendency to underestimate the true mutagenicity and carcinogenicity of metal ions with a high charge/radius ratio (*420*). The metabolic slowness of Al^{3+} could, however, also be a factor that prevents Al^{3+} from inducing cancer—providing that a sluggish uptake results in accumulation mainly in long-lived postmitotic cells that in general very rarely give rise to cancer.

TUBULIN AND MICROTUBULES: The similarities between the Al^{3+} and colchicine effects on neurons are, from a biological point of view, extremely interesting. If the mechanisms are truly analogous, Al^{3+} should have the capability of affecting many vital functions in the body and in the cells that have been shown to be affected by colchicine. The significance of such a "colchicine-like effect" could even be greater than the effect on chromatin and DNA, particularly with a relatively acute exposure. A discussion of all possible effects would, however, be premature—only a few examples will therefore be briefly taken up.

Neurofibrillary degeneration is probably an indication of a very powerful Al^{3+} influence on neurons. In smaller amounts, Al^{3+} could inhibit axonal flow (*546*) in the same way as colchicine and thereby inhibit the transport of transmitter substances to nerve endings, or enzymes for their synthesis.

In high concentrations, the effect of Al^{3+} (*182*) and Be^{2+} (*537,538*) on cell division were similar to the colchicine effect. Colchicine and other meiotic and mitotic inhibitors can, in a concentration that is too low to induce complete inhibition, instead induce nondisjunction and aneuploidy (*547*). Possibly, Al^{3+} could have the same effect.

Colchicine has been shown to inhibit secretion from a large number of endocrinal and exocrinal glands. If Al^{3+} could do the same, then it would be possible that Al^{3+} could also produce toxic effects in this way and perhaps disease.

How the reported effects on tubulin and microtubules (*206*) are induced has not been investigated, but the documented close similarities between Al^{3+} and Cr^{3+}, biochemically and metabolically, might suggest a possible mechanism.

1. When tubulin is polymerized to microtubules an equimolar amount of Mg^{2+}–GTP is incorporated, part of it without being hydrolyzed. It has been shown that normal microtubules, as it would seem, can be assembled even if Mg^{2+}–GTP is replaced by Cr^{3+}–GTP (*548*).

2. The normal equilibrium between tubulin and microtubules is believed to be governed by the Ca^{2+} concentration. Tubulin has a special Ca^{2+}-binding site (*549*), and Ca^{2+} inhibits polymerization of tubulin or induces depolymerization of microtubules (*550*).

3. The incorporated nucleotide is probably exchangeable only if the microtubules are depolymerized.

4. Microtubules with Cr^{3+}–GTP, however, were shown to be more resistant to Ca^{2+}-induced depolymerization than normal microtubules (*548*), which should imply that Cr^{3+}–GTP in microtubules is less exchangeable than Mg^{2+}–GTP, and that Cr^{3+} could thereby become enriched in microtubules and displace Mg^{2+}.

5. Microtubule function depends on perpetual polymerization and depolymerization. The morphologically normal microtubules with Cr^{3+} can, therefore, be assumed to have been functionally defective.

6. If Al^{3+} could, in some manner, accumulate in overstabilized microtubules that function poorly or not at all, this could hypothetically explain the "colchicine-like effect."

Is Al^{3+} Accumulated in Organisms? The question if Al^{3+}—normally or under special conditions—is accumulated in the body is essential for judging the possibilities that Al^{3+} might cause disease. The question, during recent years, has generally and categorically been answered in the negative. It is obvious that Al^{3+} is not accumulated in the same manner as the well-known accumulating heavy metal ions cadmium, mercury, and

lead. Accumulation could, however, occur at different "levels," e.g., in a nutritional chain, at the organism level, at the organ level, at the cell level, or at a subcellular level. The following discussion will treat the question accordingly.

NUTRITIONAL CHAIN: Humans and other higher animals probably ordinarily contain approximately 0.5 mg Al^{3+}/kg wet weight. Most vegetable foodstuff contains, however, 1 to 20 mg/kg, with a probable average of 3 mg/kg (*16,512*). With a normal renal function, the concentration in the human body should never reach the level present in food and, therefore, nutritional chain accumulation does not occur in humans.

ORGANISM LEVEL: No direct investigation at the organism level seems to have been performed regarding any possible accumulation of Al^{3+} in man. The only present method that could have been used, whole-body neutron activation analysis, suffers from the problem that P is partially codetermined with Al, which makes estimations at normal Al^{3+} levels far too inexact. To elucidate the problem, the best indirect method is probably to study the long-term metabolism of the various Al^{3+}-like ions. Most reported investigations have used short-lived radionuclides, but $^{46}Sc^{3+}$ is reported to have a very long biological half-life time in humans and rats (*281,285*). The necessary conditions could therefore exist for a lifelong accumulation in structures that provide the most stable binding sites. This is also verified, to some degree, by observations in humans of increasing Sc^{3+} concentrations in some organs, foremost in the liver, that seemed to be age-dependent (*286*). Also the long-term metabolism of $^{51}Cr^{3+}$ is consistent with a very long half-life time for a substantial portion of the Cr^{3+} taken up (*404,405*).

ORGAN LEVEL: The question of whether Al^{3+} accumulates, or can accumulate, in the brain is of greatest interest, since the toxic effects of Al^{3+} have concerned that organ. All published information regarding the normal concentration of Al^{3+} in the brain are based on rather small groups, and no detailed investigation seems to have been done concerning any possible age difference. However, three independent and concordant articles have reported, as incidental findings, an increase with age of Al^{3+} in the human brain from about 0.2 mg/kg wet weight in newborns to about 0.6 to 0.7 mg/kg in the elderly (*112,115,450*). A similar increase has been reported also for Cr^{3+} (*286,287*) and possibly for Sc^{3+} (*287*). Such a rate of increase is in good agreement with the above estimated very slow uptake of Al^{3+} in the brain, if—at the same time—elimination is assumed to be minimal or nonexistent. No direct demonstration or measurement of any elimination of Al^{3+} or the Al^{3+}-like ions from the brain appears to have been performed. That elimination must be very slow is shown, however, in patients who had obtained increased levels of Al^{3+} in the brain through hemodialysis and who subsequently received successful renal transplantations. These patients are reported to have had apparently unchanged, very high concentrations of Al^{3+} in the brain up to 5 years after transplantation (*123*). As a preliminary conclusion, it can be stated that the available data are fully consistent with a very slow normal accumulation of Al^{3+} at the organ level in the brain, but at present, no evidence exists to substantiate it.

Many earlier conclusions that accumulation of Al^{3+} does not occur in the brain have probably arisen from the conception that accumulation should lead to a large relative increase of the concentration. The narrow range between the normal and lethally toxic concentrations must, however, imply that only a rather limited quantity can be accumulated. If the estimations regarding the normal rate of uptake for Al^{3+} in the human brain are correct, then the toxic level should be reached after 100–150 years if no elimination from the brain occurs. Biologically, this seems to be a reasonable assumption. Accumulation of Al^{3+} in the brain could, during evolution, have become a life span limiting factor. In man, and other long-lived species, improved mechanisms for avoiding this accumulation could therefore have been selected for and as a final result Al^{3+} accumulation would no longer be a limiting factor. However, no extra "safety margin" could be developed in this way, and the toxic level should, according to this hypothesis, almost be reached after a maximal life span.

The risk, if any, for accumulation of Al^{3+} in the brain with a substantially increased Al^{3+} intake, in the form of antacids, has often been discussed in recent years. With an intake of 2 g $Al(OH)_3$, the urinary excretion of Al^{3+} increased from 16 to 300 μg/day (*34*). According to other investigations, antacids increased the average excretion of Al^{3+} by a factor 4–10 (up to almost 500 μg/day (*33*) or by a factor 8 (200 μg/day) (*552*). If absorption of antacid Al^{3+} is assumed, therefore, to be approximately 300 μg/day and if 0.15% of this is transported to the brain (see discussion above), then the "extra" brain uptake of Al^{3+} would be 0.45 μg/day or 164 μg/year. If Al^{3+} did accumulate in the brain without any elimination occurring, the extra content in the brain, as a result of antacid intake, would be 1.6 mg after 10 years, and a certain risk of poisoning could exist, according to the information in Table 10. After 20 years definite poisoning should appear. The total intake of $Al(OH)_3$ would then be almost 15 kg. This estimation, however, should be regarded as only an arithmetic example. No definite proof exists that there would be any effect on the brain or that such effects would be the first ones to appear after excessive Al^{3+} intake over long periods. It would appear that effects are more dependent on the total time antacids have been used than on the total dose received, since dose increases at this level would hardly increase the amount of Al^{3+} ions and complexes that could be absorbed by the intestine.

Very few investigations have been performed to study any age variations of the Al^{3+} concentrations in other tissues. For the lung it is well documented that there is an increase with increasing age but, at the same time, this is of limited interest (*46,103,125*). Similarly, for the other ions discussed there is an almost total lack of information. Theoretically, there is probably reason to believe that, for most organs, Al^{3+} has reached a steady-state concentration at the organ level, at least in adults.

Cell Level. In organs where Al^{3+} does not accumulate at the organ level, an accumulation could still theoretically be possible at the cell level, if the oldest cells, and therefore those containing the most Al^{3+}, are gradually eliminated and replaced by cells with less Al^{3+}. Such a process could normally be present in all organs mainly composed of mitotic cells, but would most probably be difficult to establish. The uptake and binding of Ga^{3+} and In^{3+} in the intestinal mucosa (*212*) could be one example of cell-level accumulation; the binding of the same ions in placental trophoblasts perhaps another (*520,521*). The most important indirect support for such a hypothesis is possibly the very close correlation between Cr^{3+} and Al^{3+} concentrations in various organs. As previously discussed, this entails that both the cell uptake and elimination in all organs must be almost identical for Al^{3+} and Cr^{3+}. The explanation for the identical cell uptake is probably that both ions are taken up via transferrin. Since a separate mechanism for elimination had to be exactly matched to the uptake mechanism in all cells the simplest explanation for the identical elimination would be, of course, that the uptake mechanism itself could be reversed and that the ions could easily leave the cells and create an equilibrium between all cells in the body. However, such an explanation seems less likely for two reasons. Firstly, cells labeled with radionuclides of Ga^{3+}, In^{3+}, or Cr^{3+} seem to bind the ion very stably. Secondly, there seems to be no correlation between the serum concentration and the tissue concentration of Al^{3+}. Instead, assuming that Al^{3+} and Cr^{3+} are intracellularly almost permanently bound without any elimination occurring prior to cell death, then elimination would also be fully parallel between Al^{3+} and Cr^{3+}.

SUBCELLULAR LEVEL: Two structures in a cell, the lysosomes and the cell nucleus, are known to accumulate Al^{3+}. Ordinarily the trivalent ions discussed here seem to enter the cells only via the Fe^{3+} pathway and they then very rapidly reach the lysosomes and probably are effectively confined within these. Long-term storage in the lysosomes most likely serves to protect other parts of the cells. If Al^{3+} is really present in microcrystalline form, as indicated (*109,133,136,137*) and as actually occurs with Fe^{3+}, then there should hardly be any risk of damage to the lysosomes either.

In some way Al^{3+} does also (probably very slowly) reach the cytosol and the cell nucleus. Theoretically, this could be due to "leakage" from the lysosomes or to direct uptake by some cell membrane process. The finding that Al^{3+} is accumulated in chromatin, especially in heterochromatin, implies that it is more stably bound there than in other structures in the cytosol–nucleoplasm compartment of the cells.

Evidently, from a cell-biological point of view, any accumulation in the lysosomes or the nucleus could have very different consequences and significance. Furthermore, in the cell nucleus it could really be a subcellular level accumulation, which could take place without any increase of the total cellular content. After a period of high Al^{3+} exposure and uptake to the lysosomes, there might even, simultaneously, be a decrease of the cell content by exocytosis and a continued accumulation in the cell nucleus. On the other hand, lysosomal accumulation should be very much the same as cell-level accumulation.

As discussed above, there might also be an accumulation on a still lower subcellular level. The slow but ultimately very heavy accumulation in heterochromatin indicates that Al^{3+} is very firmly bound there, possibly even irreversibly, at least in relation to the life span of the cell. Then, there should also be a whole spectrum of bindings from easily reversible to completely irreversible. In such an environment Al^{3+} should, according to the earlier discussed model, tend to move slowly towards the more stable binding sites. The last phase in this process could be so extremely slow, however, that the final equilibrium within each separate cell nucleus, ultimately leading to saturation of its heterochromatin, would never be reached.

General Conclusions

Al^{3+} has in recent years been discussed as a possible health risk (*553*). The interest for its metabolism has therefore increased, but one main problem exists—that Al lacks suitable radionuclides for biological studies. The working hypothesis for this review has been that comparison of the metabolisms of Al^{3+} and several similar ions could elucidate certain essential, but hitherto unsolved metabolic problems regarding Al^{3+}. Consequently, Ga^{3+}, In^{3+}, Sc^{3+}, Y^{3+}, Be^{2+}, Zr^{4+}, Cr^{3+}, and Fe^{3+} have been discussed in connection with Al^{3+}. No previous comparative review in this field could be found in the literature.

With the exception of Fe^{3+}, knowledge about the metabolisms of the ions is very fragmentary. Fe^{3+} also differs metabolically in many ways from the other ions, which as a group show a large number of well documented similarities and very few differences. Particularly strong—and hitherto unknown—similarities have been found between the best investigated ions Al^{3+} and Cr^{3+}. The comparison has, therefore, confirmed the earlier vague knowledge and conceptions regarding the metabolism of Al^{3+} and has shown that radionuclides of Ga, Sc, and Cr probably can be used as substitutes to investigate the metabolism of Al^{3+}.

Based on documented direct observations of Al^{3+} and a number of analogies from the other ions, a hypothetical model is presented for the metabolism of Al^{3+}.

As Al^{3+} is neurotoxic, its metabolism in the brain is of great interest, and it is noted that the normal and lethally toxic brain concentrations of Al^{3+} are documented by more than 15 reports. These investigations are in good agreement and imply that the lethal concentration exceeds the normal level by only a factor of 3–10. Other reported observations indicate that the Al^{3+} concentration in the brain increases during life. The estimated normal uptake of Al^{3+} in the brain (based, in part, on the normal intestinal absorption of Al^{3+} and the brain uptake of intravenously administered radionuclides of the other ions) is consistent with a model, according to which Al^{3+} once taken up by the brain cannot be eliminated

and therefore is accumulated. The estimated normal uptake is such that the toxic level in humans should then be reached in 100 to 150 years.

Cell uptake is probably very sluggish and occurs most likely only from transferrin bound Al^{3+}. Large cells are presumed to take up more than small cells. Within the cells, Al^{3+} accumulates first in the lysosomes but a very slow transfer to the cell nucleus then seems to occur. There are several reasons to assume that the reviewed ions can leave the cells, after uptake, only to a very limited extent and this should apply especially to cytosolic and nuclear compartments. The ions should, in this case, have a tendency to accumulate intracellularly. In organs composed of postmitotic cells this should, therefore, lead to an increase of the Al^{3+} concentration, but in other organs a steady state should be reached between the Al^{3+} accumulation and the elimination of dead cells, that were replaced by cells with a lower Al^{3+} content. Therefore, the greatest risk for Al^{3+} affecting the cells should be found in large long-lived postmitotic cells.

In the cell nucleus, Al^{3+} is probably mostly bound to heterochromatin by replacing, or displacing, Mg^{2+} as a counterion to phosphate groups. Al^{3+} can be assumed to have a stabilizing effect on the supernucleosomal chromatin structure and thereby also have a condensing effect on euchromatin. Al^{3+} has the ability to crosslink different polyanion chains which has been practically used in the tannage of leather by Al^{3+}. After experimental Al^{3+} poisoning, Al^{3+} has been found in neuronal heterochromatin in comparable concentrations to those found in tanned leather. It is hypothesized that Al^{3+} accumulation, by this mechanism, could be one of the causes of normal cell aging.

There are also grounds to believe that Al^{3+} can affect the microtubular function in cells in a way that resembles the effect of colchicine. Therefore, Al^{3+} might affect many cell functions of vital importance.

Al^{3+} forms stable complexes with ATP and also probably many other organic phosphate compounds. *In vitro*, Al^{3+} has been shown to inhibit several biologically important enzymes and membrane transport systems. The current knowledge in this field is, however, too sparse to allow a conclusion if this can also occur *in vivo*.

Does Aluminum Cause Disease? A Study of Possible Implications of Current Biochemical and Metabolic Concepts

It has become generally accepted that Al^{3+} may cause intoxication and disease in some rather artificial situations, e.g., chronic hemodialysis and heavy occupational exposure to Al-fumes (welding) or dust of soluble Al^{3+}-compounds. During the last decade, a few groups have reported connections also between Al^{3+} and senile dementia (and some related disorders). These connections have not seemed quite conclusive, however, and the general opinion has apparently been that Al^{3+} is very atoxic and does not cause disease under natural conditions. As an illustration of this consensus a recent, large review (*553*) on the health effects of Al^{3+} barely mentioned the existence of these reports and did not comment on them (see also note 15).

Against this background there may seem to be no substratum and no need for a review on the present topic. However, this section is not strictly a review; instead, the intention is to point out and discuss more broadly some possibilities that have occurred to the author during the work on the above review on the biochemistry and metabolism of Al^{3+}. Hopefully, the discussion will give an impression that Al^{3+} indeed could be a pathogenic agent worth considering in several connections. It is not claimed, however, that Al^{3+}, on the basis of the cumulated literature, is the most probable cause of any of the discussed diseases, previously not connected with it.

Diseases Commonly Accepted as Caused by Al^{3+}

Two conditions have been commonly accepted as caused by Al^{3+}. Both occurred in patients treated by hemodialysis, and they have now largely disappeared as a result of improved water purification routines. The conditions are discussed here in some detail as they have added a lot of information on the behavior of Al^{3+} in the body. However, it should be taken into account that this information could well be of limited general relevance due to the rather artificial conditions (excessive blood uptake and lack of renal excretion).

Dialysis Encephalopathy Syndrome. In the early 1970s a new type of neurologic disease occurring among patients on long-term hemodialysis was reported from several centers (*554–559*). The disease most often began with speech disturbances. Later, ataxia, dyspraxia, and increased dementia were observed; even later facial grimacing appeared, myoclonic jerks, convolutions and in many cases also epileptic seizures. The EEG pattern was characteristically altered already early in the course of the disease. Most often the disease rapidly led to death within 1 to 6 months, occasionally taking up to 18 months (*73,118,504,555–557,559–561*). The disease appeared characteristically as minor epidemics. In the beginning, the disease was referred to by several names. One of these, Dialysis Encephalopathy (Syndrome), DES, is used here. Another often used name is dialysis dementia.

Microcytic anemia was often prevalent with the syndrome (*89,461,562–565*) and a special type of osteomalacia (discussed separately below), both of which usually appeared before the neurologic symptoms (*562*). The anemia is believed not to have been caused by iron deficiency as administration of iron had no effect whatsoever and serum ferritin was often increased (*563,565*).

The published descriptions of the neuropathologic changes are remarkably brief and partly even contradictory (*89*). Two groups reported no changes at all (*565,566*). Some other investigations reported neuronal loss (*119,566,567*) particularly of Purkinje cells (*119,567*). Some groups have reported increased lipofuscin deposits (*135,567,568*), some an increased occurrence of corpora

amylacea (*557,567*), some spongiform changes in the neuropil (*566,567*) and some the occurrence of lacunar infarctions (*504,558*). Some studies have reported an increased occurrence of neurofibrillary degeneration (*135,566*) and senile plaques (*566*); others have denied such changes (*567,568*). One of the more richly detailed descriptions also reported the occurrence of cellular and nuclear shrinkage with increased nuclear stainability (*567*).

REPORTED Al^{3+} STUDIES: Even before DES was described as a clinical condition (see note 16), high concentrations of Al^{3+} had been detected in the serum and skeleton of uremic patients who had received Al^{3+} hydroxide to reduce intestinal phosphate absorption (*61,569,570*). The first hypothesis that DES could be an Al^{3+} poisoning came from the same group that had first described the actual clinical condition (*122*). The hypothesis was based on a comparison of the Al^{3+} concentration in muscle, bone tissue, and the brain of humans who died with a normal renal function or after long-term hemodialysis, with or without signs of DES. Dialysis patients not suffering from DES were found to have concentrations of Al^{3+} in the brain three times those found in normals and patients with DES levels about ten times those in normals. Similar differences were detected in muscle and bone tissue and the concentrations found in all three tissues showed a statistically significant correlation to the duration of chronic dialysis.

Other reports have later confirmed that blood and tissue concentrations of Al^{3+} are significantly increased with DES (*23,73,96,102,113,118,119,123,461*) in comparison to those found in normal individuals and in dialysis patients without DES. However, the differences observed could possibly be explained by the DES patients generally having received dialysis and oral Al^{3+} hydroxide for longer periods of time than patients not suffering from the disease. High tissue concentrations of Al^{3+} could simply be a marker for a long disease duration. One investigation showed, however, only for patients without DES, a significant correlation between the Al^{3+} concentration in the brain and length of dialysis treatment. Patients suffering from DES had a higher concentration. According to the authors, the absence of any correlation was due to the fact that all DES patients, after varying periods of time, had accumulated approximately the same, lethal, quantity of Al^{3+} (*23*).

Several investigations have studied the Al^{3+} concentrations of the water used for the dialysis. Patients with DES had been exposed to higher Al^{3+} concentrations than patients without DES (*102,118,461,505,561,571*). Al^{3+} is commonly used in water purification when no natural ground water is available and poor quality, perhaps contaminated, surface water is used. Therefore, the differences could theoretically imply that the poor quality water contained toxic substances that induced DES, and the Al^{3+} found was only a marker for the poorer surface water used. Some investigations, however, showed strong contradictory evidence. Two dialysis centers in the same town used the same main's water. At one of the hospitals, six cases of DES occurred during a 6-month period, at the other hospital none. The first hospital had equipped a stainless steel water storage tank with an Al anode to protect it from corrosion and this arrangement resulted in a 15-fold higher concentration of Al^{3+} in the dialysate (*102*). Moreover, two large multicenter studies, each including about 1300 patients on regular dialysis, showed that the incidence of DES correlated significantly to the Al^{3+} level of the dialysates (*571*) or the cumulated Al^{3+} exposure by hemodialysis (*561*). The accumulated evidence, therefore, is considered to have clearly shown that high concentrations of Al^{3+} in the dialysate was an important etiologic factor in the DES cases studied. Installing special water purification units at DES-affected centers has also caused DES epidemics to cease (*46,86,504,560*).

Table 12. Duration of dialysis before appearance of DES symptoms in relation to Al^{3+} concentration of dialysate; exerpts from the literature.

Number of cases	Duration of dialysis, yr	Dialysate Al^{3+}, μg/L	Reference
8	6 (3–10)	approx. 75	(*23*)
9	5.6 (2.0–8.8)	110 ± 50	(*563*)
20	2 (1–3.5)	200 (100–400)	(*504*)
11	1.7 (0.3–2.4)	140	(*86*)
6	2.4 (1.5–3.3)	approx. 1000	(*102*)
14	3.6 (1.3–8)	180	(*119*)
11	<4	150–800	(*505*)
8	<1 (0.25–3.3)	640	(*560*)
13	3.1 (1–4)	150–300[a]	(*73*)
14	2.7 (1.5–4.5)	200–800	(*118*)

[a] Occasionally > 1000.

The concentration in the brain of deceased DES patients was 1 to 8 mg/kg wet weight, average level 4 mg/kg, which is in agreement with reported lethal levels in animal experiments (Table 10). If the normal Al^{3+} concentration is accepted to be 0.5 mg/kg wet weight (Table 4), the values found in DES represent an 8-fold increase. In other tissues, a considerably greater increase has been found; in the liver 73-fold, in the spleen 189-fold, and in the skeleton 85-fold (*23*). The total Al^{3+} body load was, at most, 3 g (*572*), which is in good agreement with other estimations (*23*). The average total Al^{3+} content of the brain was 5 mg or 0.17% of the body's total Al^{3+} load (brain weight is roughly 2% of the body weight).

In the liver, Al^{3+} was detected almost solely in the hepatocytes, mainly in the large lipofuscin-filled lysosomes (*136*). Kupffer cells contained considerably smaller quantities. Also in the neurons, Al^{3+} was found in the lysosomes (*138*), and the concentration was estimated to be about 1%. Small microcrystals were detected and believed to consist of crystalline Al^{3+} phosphate.

At dialysis centers where DES occurred, the first symptoms were generally observed when patients had been on regular hemodialysis for 2 to 3 years (Table 12). Probably, they had then received some 300 treatments. If the Al^{3+} load was obtained entirely by dialysis, then each treatment might have added about 10 mg. However, part of the Al^{3+} may have originated from gastrointestinal absorption of Al^{3+} hydroxide; therefore, the additional load from each treatment may have

been somewhat less.

Al^{3+} kinetics during hemodialysis has also been directly studied in humans (*56*) and dogs (*52*). These and other investigations showed that the plasma concentration increases during dialysis. Despite considerably higher concentrations in plasma compared to the dialysate, Al^{3+} was easily transferred to the plasma. Samples were drawn from the entry and exit ports of the dialysis filter, and from the concentration gradient between these samples and the plasma flow rate through the filter, the uptake of Al^{3+} could be estimated directly. For a dog weighing roughly 20 kg, it was 2 to 2.5 mg during a 4 hr dialysis with an Al^{3+} concentration of about 75 μg/L in the dialysate. The value appears quite compatible with the above estimated uptake.

Both for humans and dogs the uptake was greatest in the initial phase. The plasma concentration increased at first but then leveled off at around 200 μg/L after about 1 hr of dialysis. This steady state seemed in part to be due to a simple equilibrium appearing between plasma uptake in the filter and deposition in the tissues. The authors suggested a saturation of the binding capacity of the plasma proteins to be a possible reason (*56,102*).

As mentioned above, the dialysate concentration of Al^{3+} and the cumulated exposure by dialysis influenced the occurrence of DES, but additional factors must exist (*561*). The higher the concentration to which a patient was exposed, the less cumulative Al^{3+} was tolerated before death. On the average, patients with DES had been cumulatively exposed to about 10 g. If it is accepted that the body load was 3 g (see above), then about 30% of dialysate Al^{3+} had been taken up. However, there was no simple relation between occurrence of DES and the cumulated exposure; many patients exposed to more than 10 g showed no signs of disease, while others, exposed to less than 5 g, clearly had DES. The chemical form, of the Al^{3+} present, was probably important.

During the 1970s, when Al^{3+} in the dialysate was found to be the primary cause of DES, it was also suggested by several workers that oral administration of large quantities of Al^{3+} hydroxide could be a contributing factor (*23,119,122,573*). After introduction of effective purification of the water for preparation of dialysate, it has been shown that plasma Al^{3+} concentrations do not increase in any appreciable degree during dialysis. Despite this, patients had considerably increased plasma levels compared to healthy individuals. This was suggested to be due to intestinal absorption of Al^{3+} (*45,52,96*). When a very low Al^{3+} concentration in the dialysate was attained (0.1–0.3 μmole/L), a significant correlation was found between the plasma Al^{3+} concentrations and the administered doses of Al^{3+} hydroxide (*45*). There have even been reports of a DES-like disease with greatly increased plasma Al^{3+} concentrations, where the dialysates used were believed not to be involved (*74,96,574,575*) and in at least 14 pediatric cases, no dialysis had occurred at all (*506,574,576–580*).

COMMENTS: The rapid transport of Al^{3+} from the dialysate to the plasma, apparently against the concentration gradient, is easily explained by the ion's high affinity to transferrin. The plasma concentration increased at first but then, with the conditions used, it rapidly leveled off at about 200 μg/L, both in humans and in dogs (*52,56*). The amount taken up in clinical dialysis should have been roughly 1 mg Al^{3+}/hr (*52,56*). If the plasma volume is assumed to have been 3 L, the circulating quantity of Al^{3+}, at any moment, would have been 600 μg. With a steady-state uptake and a plasma clearance rate of 1 mg/hr, the biological half-life time in plasma should have been 0.41 hr. At normal Al^{3+} concentrations, it is probably about 4 hr. The difference suggests that the metabolism of Al^{3+} during dialysis with Al^{3+} containing media may differ considerably from the normal metabolism. At levels of 200 μg/L, almost 30% of plasma Al^{3+} was ultrafiltrable, while at the normal level the ultrafiltrable fraction may be as low as 5%. Possibly the same conditions existed as reported for Ga^{3+} (*581*), the ultrafiltrable fraction having a very short half-life time and the transferrin-bound fraction being eliminated from plasma with the same rate as normally. If this is true, 180 μg circulating ultrafiltrable Al^{3+} (30% of 600 μg) must have been eliminated in 0.18 hr to achieve the uptake and plasma clearance rate of 1 mg/hr. The half-life time would then have been 0.12 hr, which agrees well with the observed half-life time reported when an Al^{3+} dose, that gave a plasma level of about 600 μg/L, was injected IV in dogs (*52*).

The plasma steady-state level of 200 μg/L in dialysis (*52,56*) is also interesting from another viewpoint. The concentration is equivalent to 7.4 μmole/L. If Al^{3+} is bound to transferrin, then the unused iron binding capacity, UIBC, should be responsible. In humans, this is about 40 μmole/L but is strongly influenced by the release rate of Fe^{3+} from the reticuloendothelial system and the uptake rate in the bone marrow. If Al^{3+} has a high affinity to transferrin, one should expect a steady-state level in the range of 40 μmole/L (1000 μg/L) in plasma. Instead, the steady state occurring at the 200 μg/L level—ultrafiltrable Al^{3+} included—indicates that the UIBC was low in the reported cases (see note 17).

Despite overwhelming circumstantial evidence pointing to Al^{3+} as the cause of DES, some authors have considered the etiology as still not being fully elucidated (*113,561,562,582*). This has not been due to a denial of the role of Al^{3+} but to the confusing fact that the disease has only affected some patients at a given dialysis center while others, similarly exposed, have lacked symptoms (*562,583–585*). According to this opinion this indicates the existence of an important etiologic factor determining the individual sensitivity to Al^{3+}. Another confusing fact has been the long silent period before any symptoms have appeared and the subsequent rapid course of the disease. However, both these circumstances could be explained by assuming that Al^{3+} uptake during the silent phase had conditioned some mechanism that, once triggered, caused a markedly increased transfer of Al^{3+} to the brain without any changes having occurred in the dialysis routines. Some other conditions also seem to agree better with a subacute toxic effect than a slow progressive accumulation of Al^{3+} over several years. Examples of such

conditions are the common occurrence of early symptoms being intermittent and occurring in close connection with the dialysis treatment, the localization of the main part of neuronal Al^{3+} to the lysosomes (*138*) and not to the cell nuclei (*106*) and the uncharacteristic, pathologic picture, particularly in comparison with that occurring in senile dementia of Alzheimer's type.

Binding of Al^{3+} to transferrin or to the skeleton probably reduces the rate of transfer to the brain. Both these bindings could also develop trigger mechanisms and an example of one, caused by transferrin, is outlined here. The other is described in the section following.

As previously mentioned, microcytic anemia without any signs of iron deficiency has been associated with DES and has often preceded the neurologic symptoms. In addition, Ga^{3+} and In^{3+} poisoning have produced anemia (*213,275*), and with In^{3+} it has been shown that the transferrin complex is bound to the reticulocyte transferrin receptors without any In^{3+} being incorporated in the cells (*261,264,265*). This binding evokes a hindrance, however, for subsequent Fe^{3+} incorporation (*265*). Possibly, this effect might arise also with Al^{3+} and could be the reason for the anemia. Irrespective of the initiating mechanism for the anemia, it should have resulted in a large quantity of Fe, previously bound in the circulating erythrocytes, being transferred to the iron depots in the reticuloendothelial system. Observations of increased serum ferritin concentrations support this assumption. Increased serum ferritin and increased quantities of depot Fe^{3+} normally lead to increasing iron saturation of transferrin, resulting in a greatly reduced UIBC. Appearance of the anemia could thereby result in the free binding capacity of transferrin gradually becoming less and less. Eventually the capacity would be exhausted—first during the actual dialysis treatment and then, possibly, even in the periods between them. If, at the same time, the skeleton's Al^{3+}-binding capacity would be exhausted, by another trigger mechanism proposed below, the result could, possibly, be an increased flow of low molecular weight Al^{3+} complexes to the brain. No account of the Fe^{3+} saturation of transferrin in DES seems to have been published (which may be due to high saturation normally not being regarded as pathological). Still, very likely, it may have been examined in many cases, making it possible to verify this hypothesis.

Summing up, Al^{3+} has clearly been the ultimate cause of DES, but the pathogenic mechanisms are very poorly understood. Nevertheless, much indicates that the Al^{3+} metabolism in DES may be very different from its normal metabolism. DES might therefore be a poorer model for other possible Al^{3+}-induced diseases.

Dialysis Osteomalacia. Patients with chronic renal failure have a tendency to develop renal osteodystrophy. Some patients instead develop osteomalacia characterized by a strongly retarded mineralization o newly formed bone tissue.

Osteomalacia can be induced also by vitamin-D deficiency and primarily that was assumed to be the cause of the dialysis osteomalacia, DOM, as the kidneys are responsible for an important step in the activation of the vitamin. However, vitamin D did not improve most patients with DOM (*586*) and the plasma activity of alkaline phosphatase was not increased, as is normally observed in vitamin D deficiency (*587*). This specific type of vitamin D-resistant osteomalacia is discussed in the present section. For more information see recent reviews (*89,588–591*).

Clinically, DOM is characterized by skeletal pain and a strong tendency for fractures (*505,562,571,589,592*). It has, epidemiologically, had a clear connection to DES (*73,86,119,505,562,567,571*). Histopathologically, DOM has been distinguished by a large increase of osteoid (unmineralized bone matrix), a reduced amount of mineralization front and signs of a strongly reduced mineralization rate (*24,502,589,593–595*).

REPORTED AL^{3+} STUDIES: Already 50 years ago it was shown that oral administration of large quantities of Al^{3+} salts (and also salts of Be^{2+}, Fe^{2+}, Mg^{2+}, and Ca^{2+}) inhibited intestinal absorption of phosphate in different experimental animals and then induced a phosphate deficiency that resulted in rickets or osteomalacia (*588,596*). It was later observed in humans that excessive intake of Al^{3+} hydroxide as an antacid caused a negative phosphate balance (*596*). Despite release from the skeleton, a progressive decrease of the plasma phosphate occurred. Experiments in animals using ^{32}P confirmed the drastic reduction of the intestinal phosphate absorption when Al^{3+} sulfate was given at the same time, but also indicated that oral administration of Al^{3+} interfered with phosphate metabolism, even when phosphate was administered intraperitoneally (*28*).

The inhibition, by Al^{3+}, of phosphate absorption led to the early general use of Al^{3+} hydroxide to prevent phosphate accumulation and thus to prevent the common form of osteodystrophy in patients with renal failure. It was very plausible, therefore, to assume that DOM could be due to excessive use of phosphate binders (*119*). The osteomalacia showed, however, no correlation to the use of the phosphate binder. Furthermore, most patients had normal plasma phosphate levels (*562,590,595*) and the condition was resistant not only to vitamin D but also to phosphate added in the dialysate, which should have normalized any possible phosphate deficiency (*69*).

Patients with DOM had generally been dialyzed for longer periods than patients lacking skeletal symptoms and had a higher concentration of Al^{3+} in the skeleton (*570*). The Al^{3+} concentration also showed a significant positive correlation to the amount of osteoid (*594*) but correlated negatively to the bone's mineral content (percent ash) (*597*) and to the bone-apposition rate (*571*). These correlations alone did not prove, however, that Al^{3+} induced DOM. Namely, one possibility could be that osteoid accumulated Al^{3+}—that was therefore present in an increased amount—and that the other correlations were secondary. However, rats given Al^{3+} intraperitoneally developed osteomalacia with an increased Al^{3+} concentration in the skeleton of the same magnitude as in DOM (*593,598,599*). Furthermore, several histochemical investigations (*594,598,600–602*) and investigations with X-ray fluorescence or secondary ion mass spectros-

copy (*24,600,602–604*) concordantly showed that Al^{3+} is almost completely localized to the actual mineralization front, i.e., to the interface between osteoid and mineralized bone. The Al^{3+} concentration in the bone in DOM and DES is reported to have been about 100 to 500 μg/g dry weight (*102,502,593,594,603,605*). According to one investigation, the concentration in the actual mineralization zone was about 5 mg/g dry weight (*603*).

The risk of developing osteomalacia was considerably greater if either untreated or softened water was used for the dialysate than if completely deionized water was used (*69*). The incidence of DOM showed a distinct correlation to the Al^{3+} concentration in the dialysate (*571*). DOM was also very common at the hospital where DES was induced by the release of Al^{3+} from an Al anode. This, together with other observations showing a decrease or disappearance of DOM after the introduction of water purification (*86,102*) is therefore considered to show that DOM has been caused chiefly by a too high Al^{3+} concentration in the dialysate.

COMMENTS: The structures that bind Al^{3+} in the skeleton are completely unknown. The ion has a high affinity to collagen and the polysaccharides that are integral parts of osteoid. Still the binding occurred only in the actual mineralization zone. This probably implies that Al^{3+} was bound to other ligands, specific to these zones. The accumulation evidently blocked the mineralization process, which indicates that the ligands can have been structures important for the mineral deposition or nucleation. According to two research groups, there are indications that once blocking occurs at a site this permanently prevents further mineralization there (*593,603*). The reported concentration of Al^{3+} in the mineralization zones is of the same magnitude as in neuronal heterochromatin after experimental intoxication and only a factor 2–3 lower than in Al^{3+}-tanned leather.

Possibly, alkaline phosphatase could be involved in the blocking of osteoid mineralization. Al^{3+} and several of the Al^{3+}-like ions are powerful inhibitors of this enzyme that is present in a particularly high concentration in the mineralization zone and is assumed necessary to produce the supersaturation with orthophosphate needed for the mineral deposition. A hereditary deficiency of this enzyme (hypophosphatasia) also results in severe vitamin D-resistant rickets and osteomalacia. A nonelevated serum concentration of alkaline phosphatase has also been characteristic for DOM. However, even if inhibition of this enzyme could explain the decreased mineralization, it would probably be inadequate to explain the actual accumulation of Al^{3+} in the mineralization zones (see note 18).

If such large amounts of Al^{3+} are bound to structures fairly specific for the mineralization zones, then it is possible that the binding results in the saturation of the specific ligands and that the inactivated, former mineralization zone cannot subsequently bind additional Al^{3+}. DOM also resulted in a reduction of the amount of active mineralization front. Taken together, this could well mean that the skeleton tends to become saturated with Al^{3+}, and this could create a trigger mechanism similar to the transferrin binding proposed above. By overloading one of these binding mechanisms the other would become more burdened. Overloading both might cause a rapid influx of low molecular weight Al^{3+} complexes to the brain. In fact, the hypothesis that overloading the skeleton's Al^{3+}-binding capacity might be a contributing cause of DES has been forwarded earlier (*23*), but the above suggested trigger mechanisms appear not to have previously been discussed.

In summary, Al^{3+} clearly has caused DOM but, on a molecular level, the pathogenic mechanisms are completely unknown.

Table 13. Probable features of hypothetical diseases by Al^{3+} accumulation. The features have been derived from the biochemistry and metabolism of Al^{3+}.

Worldwide occurence with a fairly constant incidence geographically and in time[a]
Possible endemic occurrence with extreme Al^{3+} exposition
Epidemiological associations between different Al^{3+}-related diseases
Lack of grave symptoms before the end of the normal reproductive period
Increasing incidence with increasing age
Possible higher incidence in males[b]
Insidious onset and slow progressive course
Similarities to normal aging processes—clinically, pathophysiologically and morphologically
Occurrence mostly in organs having large, long-lived postmitotic cells, especially in CNS
Possible accumulation of Al^{3+} only on a cellular or subcellular level
Not necessarily similar to acute or subacute poisoning caused by artificial administration
Possible similarity to conditions caused by other metal ions

[a] Hereditary disposition and incidence differences between races might be possible.
[b] Provided that published reports regarding secretion of Al^{3+} in milk are correct.

Diseases with Proposed Al^{3+} Connection

The above review on the biochemistry and metabolism of Al^{3+} and its related ions was made with the purpose of finding support for or against the existing hypotheses that Al^{3+} causes or contributes to some specific naturally occurring diseases. It appeared that increased knowledge concerning the metabolism and potentially very powerful biological effects of Al^{3+} could be used to deduce the general features of hypothetical diseases caused by it. A somewhat speculative attempt to such a deduced description is presented in Table 13. With reference to this table, the two diseases that originally initiated the above review will be discussed in this section.

Senile Dementia of Alzheimer's Type. Alzheimer's disease is poorly defined. Many still use the eponym to denote a comparatively unusual form of presenile dementia with onset before 65 years of age. Others include many cases of the more common senile dementia, and this usage appears to be gaining acceptance as both forms of the

disease show the same clinical, neuropathologic, and histochemical findings (*606–609*) and age distribution does not indicate the existence of two forms (*610,611*). The wider sense is often emphasized by the term "senile dementia of Alzheimer's type" (SDAT), also used here. The disease is defined chiefly by its neuropathologic findings (see below). However, changes associated with SDAT mainly differ quantitatively from those associated with normal aging (*609*). Identical or very similar changes are found in diseases belonging to the "Guam complex" (see below) and very great similarities also exist with other forms of amyotrophic lateral sclerosis and Parkinson disease. Milder or more severe forms of these diseases also often occur together with SDAT. Therefore, several authors have recently suggested that all these diseases should be regarded as one entity where the various forms represent a dominating distribution within different areas of the nervous system (*612–614*).

Clinically, the disease is characterized by a progressive deterioration of memory and intellect (see note 19). In the beginning, the course is insidious and the patient often lacks insight of disease. The symptoms are very uncharacteristic and difficult to distinguish from other types of brain failure or from symptoms of normal aging. Patients with SDAT have a considerably increased mortality compared to healthy individuals of the same age, and the entire course of the disease takes, on the average, about 8 years with a range of 2–20 (*608,615*).

Due to the vague distinction towards normal aging and the difficulties associated with establishing the diagnosis, the epidemiologic characterization of the disease is still incomplete. However, SDAT is generally believed to be the most common cause of brain failure in the elderly (*615–617*), and the disease is stated to be the fourth or fifth most common cause of death in the U.S. (*617*). SDAT seldom appears before the age of 40, but its incidence and prevalence subsequently increases with age. Some authors have stated that SDAT is more prevalent in women than in men, but this is believed, by others, only to be due to a predominance of women in the most afflicted age groups (*608,610,618*). SDAT is said to occur with about the same prevalence throughout the world (*610*). A certain genetic predisposition is believed to exist, and SDAT shows some connection to the HLA system (*619–622*). Most cases occur, nevertheless, entirely sporadically, and therefore the genetics are unknown. In certain cases, however, SDAT is inherited as an autosomal dominant with a high penetrance (*623,624*). A detailed investigation of a large family, with 47 cases affected by this form of SDAT, suggested the presence of two genes, one localized to the HLA region on chromosome 6 and one probably close to the Gm region on chromosome 14 (*621*). A genetic connection with Down's syndrome and with familial leukemia has recently been reported (*625–628*). This relationship has lead to a hypothesis of a primary microtubular defect (*623*).

Reported neuropathological changes comprise mainly neuronal depletion and general cell atrophy of the brain, reduction of the "dendritic trees," changes in the cell nuclei, neurofibrillary degeneration (NFD), senile plaques, amyloid angiopathy, granulovacuolar degeneration and the presence of so called Lewy and Hirano bodies (*606,607,609,615,618,629,630*). The changes occur, to a varying degree, in the entire CNS, but are said to be most pronounced in the hippocampus and in certain parts of the neocortex (*606,618,631*). The cell loss chiefly affects the largest neurons (*632–634*), and in histologic specimens it is easily underestimated as packing of the remaining cells occurs.

No distinct picture exists of the pathophysiological mechanism operating, nor any knowledge of which changes are primary or how the different changes relate to one another. According to one hypothesis, a disturbed intracellular transport of proteins could be the most important factor. The disturbance would, among other things, result in various proteins being accumulated in the vicinity of the cell nucleus instead of being transported to the cell periphery. Deficiency of proteins for neurofibrils and membranes of the dendrites and axons would subsequently result in degeneration and atrophy, loss of synaptic functions and finally destruction of the whole neuron. This hypothesis is, as yet, essentially unconfirmed and it may, possibly, also partly be based on comparisons with changes occurring in experimental Al^{3+} poisoning.

The primary change in SDAT is unknown. One group has demonstrated, in neurons from the temporal cortex, a reduction of the nucleolar volume in cells containing NFD (*635*). Another group has reported the reduction of the nucleoli to be greatest in neurons with NFD (*636*) but present also in neurons without NFD and this nucleolar reduction correlated well with the occurrence of NFD and senile plaques in the same region (*637*). This was believed to imply that the nucleolar changes had occurred earlier than the other two changes. The nucleolar size is considered, in general, to correlate closely to the rate of RNA and protein synthesis in the cells. In SDAT the neuronal RNA content was reduced, on the average 26% (*638*), and the greatest reduction (30–50%) was observed in the hippocampus and nucleus basalis (*639,640*). The mean volume of the cell nuclei was also reduced (average 43%), and signs were believed to exist that this change occurred prior to the reduction of the nucleolar volume and RNA content (*638,641*). Another group has reported the RNA content only to be reduced in neurons with NFD (*642,643*).

In SDAT patients, chromatin of neurons and glial cells is reported to exist as heterochromatin to a greater extent than in healthy controls [on the average 44.4% (22–74%) compared to 24.8% (± 5.5%, SD)] (*644*). The chromatin was also more resistant to micrococcal nuclease, presumably, according to the authors, due to its more compact conformation (*645*).

NFD and senile plaques are the foremost recognizable neuropathologic signs in SDAT. NFD consists of tangles of protein fibrils, in typical cases observed in 1 to 10% of the neurons, mostly the large ones (*615*). NFD is common near the nucleus, but is also found in dendrites and even in nerve endings. In the electron microscope, the fibrils differ from the NFD caused by Al^{3+} poisoning in

animals mainly by being "paired helical filaments" (PHF). The same type of NFD is also seen in other chronic neurologic diseases, and to a lesser extent even in healthy old people (*646–648*). Similar PHF (with a slightly lower pitch) has been observed in aging rhesus monkeys (*649*) but has not otherwise been observed in other species (*650*). The structure of PHF is not known for certain. Possibly, both tubulin and proteins from the normal neurofilaments are part of its structure (*629,651–658*). According to one report, isolated PHF are insoluble in the presence of urea, sodium dodecyl sulfate or reducing agents which can possibly indicate the presence of covalent crosslinkage (*659*).

It is not known if NFD *per se* damages the neuron or if it is merely a marker for a deeper rooted damaging process. NFD is believed to develop very slowly and, therefore, an affected neuron is assumed to survive for many years although with considerable changes progressively occurring (*629*). Possibly a neuron cannot eliminate the PHF tangles.

Senile plaques are rounded structures, 10 to 150 μm, consisting of amyloid fibrils surrounded by glial cells and degenerated axonal nerve endings (*615,630*). They are often localized close to small blood vessels (*607,630,660*). No clear picture of their origin has been found (*661*), but interest has mostly been focused on the amyloid. Several authors have associated senile plaques with amyloid (or congophilic) angiopathy, which is also regularly seen in SDAT, and is recognized by the presence of amyloid in the basement membrane of the capillaries and small arterioles (*662,663*). Senile plaques are believed to develop extremely slowly (*629*).

The third and least known neuropathologic cardinal sign in SDAT is granulovacuolar degeneration. This consists of solitary or groups of membrane bound vacuoles, 2 to 5 μm, in neuronal cytoplasm, each containing a fairly electron-dense hematoxylinophilic and argyrophilic granule, 0.5 to 1.5 μm (*606,607,664,665*). This form of degeneration has been recognized for just over 70 years; despite this no knowledge of its composition, how the changes are induced or its life cycle exists (*606,607*). However, certain circumstances indicate a slow progressive process (*665*). The changes are considered suggestive of abnormal endocytotic vesicles or lysosomal residual bodies (*607*). Granulovacuolar degeneration is reported to be observed in nearly all individuals with SDAT and is believed to be most developed in the pyramidal cells in the hippocampus, where it is reported in 9 to 66% of the cells (*664,665*). The findings occur to a lesser degree in elderly individuals without dementia.

A large number of investigations have demonstrated neurochemical changes in various parts of the brain in SDAT. The concentration was reduced for most of the investigated neurotransmitters and related enzymes. The most pronounced changes were observed for acetylcholine, cholinacyltransferase and acetylcholinesterase. Therefore, SDAT is believed to be a disease primarily affecting cholinergic neurons. The common conception is that the changes observed reflect the loss of different groups of neurons and not a specific inhibition of certain enzymes.

SDAT has no commonly accepted etiology. The foremost discussed hypotheses include slow viruses, toxic environmental agents (especially Al^{3+}), and immune defects or autoimmunity (*126,609,618,641,666*). Attempts to transfer SDAT to monkeys have been made but have seldom succeeded (*624*), and the possible transfer of agents without any association to SDAT cannot be excluded. Due to diagnostic difficulties, it could even have been wrongly diagnosed cases of Creutzfeld-Jacob's disease (*667*). However, in some cases classed as familial Alzheimer's disease, the disease might have been transmitted (*668*), and tissue samples may have shown *in vitro* cell fusion activity (*669*). If this is true, these cases should be regarded as a special group. No convincing evidence indicates that auto-antibodies can induce SDAT (*670,671*). In cases where such antibodies were present, they might have been secondary to the disease.

REPORTED AL^{3+} STUDIES: During the last 10 years a Canadian group has published several investigations concerning the brain concentration of Al^{3+} in SDAT. In a first report (*552*), three individuals were reported to have increased concentrations, 1 to 10 mg/kg dry weight (mean 3) compared to 0.5 to 2.5 mg/kg (mean 1) in normal brains. It was pointed out that the concentration was of the same magnitude as in Al^{3+}-induced NFD in cats. It was concluded, therefore, that Al^{3+} could well be a neurotoxic factor in SDAT. In later, more extensive investigations (*108,672*), this group has been able to substantiate their earlier findings. On the average, investigated SDAT samples showed a (1.5–2 times) increased brain concentration of Al^{3+} compared to samples from nondemented individuals. The concentrations in different parts of the brain also correlated remarkably well with the occurrence of NFD.

Fluorescent-histochemical investigations indicated that a considerable fraction of the Al^{3+} was localized in the cell nuclei. This group, therefore, isolated neuronal cell nuclei and separated their euchromatin and heterochromatin. Whole cell nuclei from SDAT neurons were shown to have a doubly increased Al^{3+} concentration compared to normal neuronal cell nuclei (*106*). Almost all Al^{3+} was localized to the heterochromatin, that contained on the average about 3700 μg Al^{3+}/g DNA, compared to about 2000 in normal heterochromatin.

The Canadian group has summarized and comprehensively discussed their results in several articles (*71,551*). The increased Al^{3+} concentration in SDAT has also, in various ways, been confirmed by some other groups (*117,121,134,673*), but two groups have reported no detectable increase (*112,115*).

The brain concentration of Al^{3+} in SDAT was, according to one investigation, increased 1.4 times over the normal concentration (*117*) and the difference was statistically significant at the level $p = 0.01$. The investigation was performed with atomic absorption analysis. One group, that utilized neutron activation analysis, reported a mean of 1.43 times increase, but the material consisted of only three SDAT individuals (*121*). Another group, using an ion probe technique, detected Al^{3+} in senile

plaques but was unable to measure the increase (*673*); the investigation included six individuals. Yet another group used an electron probe (*134*) and studied the occurrence of Al^{3+} in the cytoplasm and cell nuclei of neurons with and without NFD. It was found that about 90% of the neurons with NFD had detectable deposits of Al^{3+} in the nuclei compared to 4 to 5% in neurons without NFD; this was true for neurons taken both from normal brains and from SDAT-brains. In SDAT Al^{3+} could be detected in the cytoplasm in 29% of neurons with NFD. In neurons taken from nondemented individuals, Al^{3+} was detected in the cytoplasm in 11% if NFD was present and in 2% if it was lacking.

The groups that reported no detectable increased concentration of Al^{3+} in the SDAT brains had both investigated a rather limited material (10–12 individuals) (*112,115*). In one of the investigations, samples were taken systematically from various parts of the brain (*112*). The average values, detected in the different areas, were almost without exception somewhat higher in the SDAT cases than in the controls (in the temporal cortex, a twofold increase). However, when all the values were evaluated together, the difference did not reach statistical significance. The other investigation was based on samples taken very much at random from the different individuals. Both investigations showed, as an incidental finding, an increasing concentration of Al^{3+} in the brain with increasing age in normal individuals.

Apparently, there are many signs pointing to Al^{3+} as being a contributing etiologic factor in SDAT. However, up to now, most authors have not considered the connection as proven (*606,609,618,674,675*) and have, for example, pointed out that Al^{3+} could accumulate terminally in already diseased neurons. Opposing this view is, however, the findings of normal Al^{3+} concentrations in the brain of many patients with Creutzfeld-Jakob's disease (*116*).

COMMENTS: If the above information and concepts from the literature on SDAT are compared with the proposed description of hypothetical Al^{3+}-induced diseases (Table 13) and with the general picture of the Al^{3+} metabolism, then it is obvious that most epidemiologic and some clinical features are well consistent with the hypothesis that SDAT may be due to a slow Al^{3+} accumulation in the brain.

The cell depletion (*632*) and NFD (*615*) in SDAT that mostly affect the largest neurons are also in agreement with corresponding findings in experimental Al^{3+} poisoning (*107,110,192,200*). The reported heterochromatization (*644*) and reduction of cell nuclear and nucleolar volumes and of RNA synthesis in SDAT-affected neurons are further examples of processes that are quite consistent with the picture of the Al^{3+}-metabolism.

The occurrence of NFD both in SDAT and in Al^{3+} poisoning in certain animals is a similarity that is difficult to evaluate, however. Obviously, the filament structures differ and possibly they are chiefly formed by different proteins. Presumably, important species differences also exist. NFD is easily produced after a fairly short-termed Al^{3+} exposure in cats and rabbits but is stated not to occur in rats (*676*) or various primates (*507*). Neither has NFD been induced in humans in dialysis encephalopathy. The time spans of Al^{3+}-induced NFD and SDAT–NFD also differ considerably. Despite the differences, it is possible that the mechanisms of origin may be common or show great similarities. Possible mechanisms might be a disturbance of the protein transport to peripheral cell parts or an altered aggregation tendency of a particular protein. Both of these changes are clearly of the kind that might possibly be induced by Al^{3+} (see note 20). Several authors have also tentatively regarded Al^{3+}-induced NFD as a model for SDAT (*551*) or at least for the NFD in this condition (*193,194,677*).

Accumulation of Al^{3+} has been demonstrated in senile plaques in SDAT (*673*). However, due to inadequate understanding of the pathogenic events, the finding gives very little grounds to judge the discussed etiologic hypothesis.

The binding of hematoxylin to the granules of granulovacuolar degeneration should imply that they are able to bind Al^{3+} and Al^{3+}-like ions (an essential requirement for hematoxylin staining). Hypothetically then, Al^{3+} might even be an integral part of the granule; this would fit neatly with the suggested lysosomal origin of the change (*607*). Perhaps granulovacuolar degeneration might be related to the lysosomes with crystalline Al^{3+}, demonstrated in dialysis encephalopathy (*138*).

In view of the narrow gap between the normal and definitely toxic concentrations of Al^{3+} in brain, the increase reported in SDAT appears quite adequate to induce serious effects. The reported Al^{3+}/DNA ratio for heterochromatin is considerable (about 1/4 of the Al^{3+}/protein ratio in tanned leather). Moreover, the nature of several of the reported pathologic changes in SDAT is also remarkably consistent with the effects these amounts of Al^{3+} could be expected to induce. In addition, no well documented fact associated with the disease or with the Al^{3+} metabolism seems to be definitely incompatible with the hypothesis. Therefore, taking all current facts into consideration, it can be stated that, indeed, many reasons exist to couple SDAT with Al^{3+}.

Endemic Amyotrophic Lateral Sclerosis and Parkinsonism-Dementia. After the second world war, the frequent occurrence of two forms of chronic neurologic disease was noticed among the inhabitants of Guam and other nearby islands. One presented itself clinically as classic amyotrophic lateral sclerosis (ALS) and the other was a mixture of parkinsonism and senile dementia, and therefore became known as parkinsonism-dementia (PD). Both conditions had an insidious onset and a slowly progressive course which normally ended in death within 3 to 5 years (*678,679*). The ALS form seldom appeared in individuals below the age of 35 years and the death rate culminated around 50 years of age (*680,681*). The PD form usually occurred about 5 years later than the ALS form (*680*). The incidence of both ALS and PD was roughly twice as high in men as in women (*680,682*). During the 1950s and the 1960s both diseases were each responsible for 15% of all deaths among adult men (*680*). Among adult women, ALS was responsible for 11% and

PD for about 8% of all deaths (*680*). For both conditions, the incidence thereafter dropped considerably (*682*). According to the spoken tradition on the island, ALS had occurred there for many generations but no direct information was available for PD. The inhabitants had not considered PD as a disease, it had no name (*680*), but had instead been regarded as aging.

After the detection of these diseases on Guam, at least two more areas, showing high incidence of the same conditions, have been found: on the Kii Peninsula in Japan (*683*) and in an area of Western New Guinea (*684,685*). In Japan, the incidence has probably been lower than on Guam and is also receding. On New Guinea, the incidence has probably been higher and the diseases have also appeared at a younger age than on Guam (*686*).

Neuropathologically, both conditions are characterized by brain atrophy with cell changes and cell depletion which, in the ALS form, have been particularly accentuated in the spinal cord's anterior horns, in the cranial nerve nuclei and in the primary motor neurons. In the PD form it has particularly been accentuated in the substantia nigra and locus ceruleus. The changes have mainly consisted of the same type of NFD as found in SDAT and of granulovacuolar degeneration. Senile plaques, on the other hand, have been rare (*687*). NFD and granulovacuolar degeneration also occur earlier and are considerably more prevalent among healthy inhabitants on Guam in comparison to healthy individuals elsewhere (*688,689*).

A lot of research has been performed which one hoped would elucidate the cause of endemic ALS and PD and also solve the etiologic problem of classic ALS. In spite of all this work, the etiology still remains unknown. The most prominent hypotheses have assumed the diseases to be hereditary, to be caused by a slow virus or an immune defect (or a combination of both) or, finally, the cause to be a toxic environmental factor.

Detailed epidemiological investigations have not been able to detect any definite hereditary factor (*690*). Since the incidence has fallen considerably during recent years and, at the same time, the onset has occurred at a later age, environmental factors may be more important than hereditary ones (*691*). Indeed, immigrants of Western origin have almost never been afflicted (*692*) but several cases have occurred among Filipino immigrants (*682,693*), which points to culturally related environmental factors.

The inhabitants of Guam are American citizens, and many have settled in California. These migrants have shown a lower incidence of ALS and PD than those remaining on Guam but a considerably higher incidence than other Americans, and cases have occurred up to 34 years after emigration (*691,694*). Those who became ill in California had all resided on Guam for more than 18 years. This, together with the observation that Filipino immigrants have fallen ill after an average of 20 years residence on Guam (*682,693*), can support the presence of a very slow acting agent.

Attempts to detect a virus or to transfer the diseases to other primates have not been successful (*695*). A report that reverse transcriptase was detected in neurons from two deceased ALS patients have so far not been confirmed by others (*696*).

Immunological tests have revealed lymphopenia (particularly of T-cells), a reduced response to mitogenic stimulation and signs of reduced cellular immunity in skin tests (*697*). Lymphocytes, from affected individuals, have not been stimulated by brain antigens from deceased affected persons (*698*).

Epidemiological studies mostly support an environmental factor, connected to the traditional life pattern, as a probable cause of the disease. A higher incidence was observed on Guam than on the other nearby islands and it was greatest in some of the most southern villages, reported to be least westernized (*682,699,700*).

One environmental factor that has received much attention is the nut from *Cycas circinalis* that grows wild on the island. Earlier, particularly in times of famine, the inhabitants have used these nuts as their principal source of carbohydrates. Cycas nuts contain several toxic and carcinogenic substances, e.g., the glycoside cycasin. No substance has, however, induced ALS-like symptoms in animal experiments (*701*). In recent years the interest for Cycas has, therefore, subsided considerably. Manganese has also been discussed as a possible cause. Mn^{2+} poisoning can induce parkinsonism. Fairly large quantities of Mn^{2+} are reported to be present in the soil and subsoil on Guam, and mining for Mn was carried out during the second world war (*679,702,703*). The concentration of Mn^{2+} in the CNS of the deceased has also been determined; some authors have reported increased values (*477,704–706*); others have found no changes (*121,707*). However, all the investigations were performed on very limited material and some of the reported changes have also been minimal with regard to the sensitivity of the methods employed.

REPORTED AL^{3+} STUDIES: In recent years, a Japanese group from Wakayama on the Kii Peninsula have published several reports regarding the concentration of Al^{3+}, Mn^{2+}, and Ca^{2+} in the brains and spinal cords from patients with endemic ALS. A first report only included four patients and three controls (*707*). The concentrations were measured by neutron activation analysis and showed fairly high normal Al^{3+} values, 17.7 ± 3.4 mg/kg dry weight. No mention is made of any steps taken to prevent phosphorus interfering with the analysis. Possibly, P can have been codetermined partly and caused the high values. However, a distinct difference was observed between the samples from the ALS cases and the controls with a twofold average increase in the samples taken from different brain areas in the ALS cases (33.7 mg/kg). The concentration was greatest in the spinal cords of the patients, about four times higher than in the controls. A later investigation added two Japanese ALS cases and four PD cases from Guam (*121*), and the results were in complete agreement with the previous investigation.

Later, by alpha particle-induced X-ray fluorescence, the group found increased concentrations of Al, Si, P, Ca, Ti, V, Mn, and Fe in ALS (*707*). Another group reported

that ALS increased the perivascular occurrence of Al^{3+} and Ca^{2+} in CNS (*477*). Only three cases were studied by using an electron probe technique. The same technique was recently used by a group which reported that Al^{3+} was detected in 58 to 68% of neurons with NFD taken from hippocampus in deceased ALS and PD patients from Guam (*139,708*), but it was detected only in 11% of neurons without NFD. Al^{3+} was mainly localized to the cell nuclei, but in many cells could also be detected in the cytoplasm.

The diseases on Guam have mostly affected people who had lived for a long time in the southern part of the island (*682*). Geologically, this part is of volcanic origin, while the remainder of the island consists of limestone. The soil in the south is described to be of "bauxite texture" with a very low calcium content, while on the rest of the island the soil has a high calcium content (*120*). Extremely low calcium contents have also been detected in those areas of the Kii Peninsula (*683,709*) and New Guinea (*685*) where ALS and PD have been found. In New Guinea, the soil is said to contain bauxite and iron oxides, and water samples showed high Al^{3+} concentrations 100 to 400 μg/L (*685*). No direct information concerning the Al^{3+} content of the Kii Peninsula soil is known, but it is reported that the Al^{3+} concentration in rice, from the area where the diseases occurred, has been higher than in rice from other areas (*710*). Finally, what may be a fourth focus of endemic ALS has recently been detected in Northern Australia and, once again, in this area there is a red Al^{3+} and Fe^{3+}-rich soil (*686,711*).

The Japanese group referred to above seems not to have explicitly designated Al^{3+} as a possible causal factor behind endemic ALS and PD. Instead, a hypothesis has been proposed that prolonged Ca^{2+} deficiency is the ultimate cause. According to the hypothesis, this results in a secondary hyperparathyroidism affecting the skeleton (see below). The hyperparathyroidism then, for some reason, becomes permanent and subsequently results in Ca^{2+} deposits in different tissues, including the CNS. Concomitantly, deposition of Al^{3+} and Mn^{2+} also increases but this has been thought of, by the group, mainly as an epiphenomenon.

COMMENTS: The increase of Al^{3+} found in the spinal cord and brain in endemic ALS appears to be of the same magnitude as found in severe Al^{3+} poisoning in experimental animals, in dialysis encephalopathy and SDAT. Therefore, it might be reasonable to discuss Al^{3+} as a possible cause for this condition too. Furthermore, the reported neuropathologic changes in endemic ALS and PD also occur in SDAT and, as argued above, are reasonably consistent with a hypothetical Al^{3+} etiology. The late onset and the slow but steady deterioration of brain functions are also consistent with a poisoning by slow Al^{3+} accumulation. This is indeed also true for the long exposure necessary for the presumed agent on Guam and for the long latent period between the exposure and the onset of illness.

In endemic ALS and PD no known cause exists for the reported reduction of T-lymphocytes with inhibition of the mitogenic blast transformation, but a clear parallel can be seen in experimental Al^{3+} poisoning (*712*) and chronic renal failure with and without dialysis (*713*).

The similar geologic conditions reported in the three or four areas with endemic ALS and PD make it hard to believe that these conditions lack importance for the occurrence of the diseases. This impression is strengthened by the lower frequency in the northern part of Guam, where the geology is entirely different (*683*). A low calcium content in the soil is usually associated with a low pH, in this case probably due to the soil's volcanic origin. A low pH and excessive rainfall probably has leached out all the Ca^{2+} compounds originally present. Bauxite is a weathering product which is mainly formed in very warm and wet climates where even silica are leached from the clays formed by erosion. Therefore, the conditions in the three (or four) areas of interest should be favorable for dissolving Al^{3+} from the soil into the surface and subsoil waters.

However, it does not appear entirely probable that the low pH and presence of Al^{3+} in the soil and water alone induce the disease. This conclusion is justified by the fact that similar conditions exist elsewhere without ALS and PD occurring, that immigrants of European origin have not been afflicted, and that in Japan and on Guam there has been a substantial decline in incidence during the last decades. On epidemiological grounds, it is also believed that some connection has existed with the traditional life patterns.

If a high concentration of soluble Al^{3+} is present in the soil, then plants must have adapted, by selection, as vegetation is reported to be abundant in all areas with endemic ALS and PD. However, this can also imply that plants contain large quantities of chelate-bound Al^{3+}, which could be absorbed in the intestinal tract more readily than less complexed salts. On the Kii Peninsula and Guam, the falling incidence can possibly be attributed to the general abandoning of locally produced food and the increased use of products from other areas.

Summing up, the pathological changes and several clinical and epidemiologic features are quite consistent with the hypothesis that Al^{3+} is a key pathogenic agent but no conclusive evidence exists and additional factors, which are probably related to the traditional way of life, must be operating. If the connections with Al^{3+} are substantiated, then the diseases may illustrate the serious environmental hazards associated with a high level of dissolved Al^{3+} in the soil.

Diseases of Unknown Etiology but with Features Suggesting Possible Al^{3+} Connection

Several aging-related diseases lack known specific etiology but show many features that compare favorably with the description of hypothetical Al^{3+} related diseases (Table 13). If it is speculated that some of these diseases were induced entirely or partly by a lifelong slow accumulation of Al^{3+}, then it might not be improbable that this connection could have been overlooked. It must be

extremely difficult to epidemiologically investigate a disease with an insidious onset, with symptoms similar to normal aging and with an etiological agent that all individuals in the world are abundantly exposed to, but which may need several decades of exposure to produce symptoms. In addition, no chemical or metabolic investigations of Al^{3+} seem to have been carried out on these diseases. Such investigations are also inherently difficult due to several particular reasons, discussed in the above review. Taken together, these circumstances have seemed to give reason for a discussion—from a sheer basic scientific point of view—of the possibility that Al^{3+} could contribute to the generation of some of these diseases. However, at the same time, an obvious argument against such a discussion has been that it could only be superficial and could appear very premature.

In addition to scientific interest, there has arisen a practical medical urgency. Al^{3+} is clearly absorbed in the gastrointestinal tract. A syndrome, apparently identical to DES, has been found in a number of patients with renal failure who were not dialyzed but had received large quantities of Al^{3+} hydroxide to reduce phosphate absorption. Symptoms appeared after only one or a few years. Against this background, it has appeared extremely important to investigate if the very widespread use of Al^{3+} hydroxide as an antacid causes any hazardous effects with long-term use or any delayed effects long after the use. At present, there are presumably no published reports that can be considered to exclude this. Possible prospective investigations are encumbered by great problems. If heavy consumers of antacids are examined, then they should be followed for very long periods, preferably for the rest of their lives, to exclude very slow-acting effects. Furthermore, the investigations should embrace a very large number of individuals, probably several thousands, if a moderate risk increase for comparatively rare diseases should be detected or excluded. Presumably, it should be easier to assess, in retrospect, if individuals with certain selected diseases have taken larger quantities of antacids than a control group. This method necessitates, however, a certain perception as to which types of diseases one should primarily investigate. Theoretical considerations and hypotheses, of the type presented, should then, hopefully, help select these.

Classic Amyotrophic Lateral Sclerosis. The term amyotrophic lateral sclerosis (ALS) is generally used to indicate a syndrome characterized by a degeneration of both the upper motor neurons in the cerebral cortex and of the lower in the medulla and spinal cord; this sense is subsequently used here. Occasionally, the disease is called "motor neuron disease" (MND). ALS is epidemiologically divided into a "sporadic" or "classic" form and a familial form, the latter representing 5 to 10% of all cases.

Classic ALS most often appears around 50 to 60 years of age, and 1.5 to 2 times more often in men than in women (*724*). The incidence in the entire population of most Western countries is said to be 1–2/100,000 per year. No effective treatment is known and classic ALS usually results in death within 2 to 5 years.

Despite the disease having been clinically well defined for almost 120 years, little is known of its etiology. The familial form is generally inherited as an autosomal dominant with limited penetrance. It also differs in other respects from classic ALS and could, therefore, represent a separate entity. Hence, familial ALS is not included in the following discussion. Since no hereditary disposition has been observed for classic ALS it has been concluded that environmental factors may be responsible; among these infection and metal exposure are most often mentioned as possible causes (*715*).

Attempts with classic ALS to detect antibodies in the cerebrospinal fluid, against a possible virus, oligoclonal immunoglobulins or changes of interferon have failed, if some isolated cases of various accidentally occurring viruses are disregarded. Metals, particularly mercury, lead, and manganese, have been suspected. In conclusion, it can probably correctly be stated that none of the hitherto published investigations have shown any of these metals to generally cause classic ALS, but they may possibly do so in certain cases. Many agents may cause ALS.

Cell depletion dominates the neuropathological changes and has primarily been detected in the spinal cord by either directly counting the neurons present in a specific spinal cord segment or by counting the fibers in the ventral root. The largest motor neurons with the thickest fibers have particularly been affected. Normally, about 5000 such cells are present in each segment (on each side). In ALS, less than half have remained, in some cases less than 100 cells (*716–721*). Cell loss of the largest sensory neurons in the spinal ganglia has also been detected (*718*).

The cytologic changes present in the remaining ALS affected neurons are reported to be similar to the changes seen in normal aging, e.g., shrinkage and pyknosis of the nucleus, reduction and deformation of the dendritic tree, and increased inclusions of lipofuscin (*722–724*). NFD and senile plaques are not normally present in classic ALS, but several intracellular inclusion bodies have been reported, e.g., Bunina and Lewy bodies (*725*). One group correlated the size of the nucleolus to the RNA content in neurons from the anterior horns of the spinal cord and the trigeminus nuclei and reported essentially the same changes that occur in SDAT, i.e., reduced RNA content and nucleolar volume (*641,723*). From the correlations, the group concluded that a reduction of the nucleoli and an increased heterochromatization are the earliest morphological changes detectable and that these lead to the reduction of the RNA content. The decrease of RNA has also been substantiated by other workers (*726,727*). Still another group recently proposed that unknown changes in DNA might render it unable to undertake transcription and thereby cause the other cellular changes (*728*).

FEATURES OF SPECIAL INTEREST: No direct Al^{3+} studies appear to have been performed on classic ALS. Patients contracting ALS have, in their earlier life, had fractures considerably more often than other individuals (*709,729,730*). The fractures occurred up to 10 years be-

fore onset of any neurological symptoms and are suggested to imply a disturbed bone or mineral metabolism. However, closer investigations of the nature of the process have not been possible because patients, when ALS was diagnosed, have often shown a demineralization of the skeleton secondary to reduced activity caused by muscle atrophy and general invalidity.

Another connection to mineral metabolism is the hypothesis, discussed above, that ALS in general (not only endemic ALS) might be caused by a calcium deficiency that leads to hyperparathyroidism and then leads to skeletal disturbances and a deposition of various cations in the CNS and other soft tissues. A slight increase of the parathyroid hormone was also reported (*120*), but other workers have found normal hormone concentrations (*731*). It is also of some interest, in this context, that hyperparathyroidism, both primary and secondary, is reported to show neurological manifestations similar to ALS (*732,733*). Finally, it may be recalled that the parathyroid hormone has been claimed to increase the intestinal absorption of Al^{3+} (*43,44*), but these reports too have been questioned by other authors (*45–47*).

Some metabolic changes have been reported in ALS, e.g., elevated aspartate concentrations in plasma and cerebrospinal fluid. The serum concentration showed a fairly close correlation ($r = 0.81$) to the disease activity (*734*). (Aspartate and glutamate are taken up by neurons, especially in the spinal cord, with a high affinity and are believed to have a transmitter function.) The serum concentrations of citrate have also been reported to be increased in classic ALS to almost twice the normal level (*735*). However, none of these findings have been confirmed by others. Aspartate and citrate are both powerful Al^{3+} chelators and, especially for citrate, are good candidates as principal complexing substances in plasma for ultrafiltrable Al^{3+}.

Two clinical ALS investigations showed an increased frequency of peptic ulcers or previous gastrectomy (*736,737*) but other investigators were not able to confirm these findings (*738–740*). A high consumption of Al^{3+} antacids could possibly have been the cause, if this connection really exists. However, as far as it is known, no systematic investigation has been performed of earlier antacid consumption by ALS patients.

One group (*741*) described special skin changes present in about 45% of the patients with classic ALS and in about 65% with endemic ALS. The changes mainly consisted of elastosis (resembling senile elastosis), increased amounts of glycosaminoglycans in the dermis, coarser, and more stainable collagen fibers, and focal degeneration and regeneration in the dermis. No pathophysiological interpretation was presented.

COMMENTS: The reported neuropathological changes, particularly the early cell nuclear changes, are distinctly reminiscent of the described Al^{3+} effects. The similarity and proposed deeper relatedness to SDAT and endemic ALS-PD provide further general reasons to discuss Al^{3+} as a possible pathogenic agent for classic ALS.

Detailed discussion of the reported fracturing tendency, the supposed skeletal changes or the hypothetical hyperparathyroidism is not possible, except for noting that some disorder of the mineral metabolism could constitute an early symptom in ALS or, possibly, be an etiologic factor.

Increased amounts of Al^{3+}-complexing substances in plasma could, probably, facilitate the transfer of Al^{3+} to the CNS. Plasma citrate is regularly increased in hyperparathyroidism and also increases with hard manual labor and physical training (*742,743*), factors that are thought to predispose for classic ALS (*729,736,744*).

The described skin changes are suggestive of the changes reported after intradermal injection of Al^{3+} in guinea pigs (*745*). Both changes were similar to those found in senile elastosis. Irregularities, coarsening, and increased stainability of the collagen were present in both as well as focal degeneration and regeneration. After the experimental administration, a slight tendency to granuloma formation and occasional giant cells were observed, but were not present in ALS. However, the changes observed in guinea pigs were subacute and appeared only 2 to 3 weeks after the administration.

In summary, several vague reasons seem to exist for connecting classic ALS with Al^{3+} and no generally accepted facts seem to argue definitely against such a hypothesis. As all other etiological hypotheses of current interest are no less vague, Al^{3+} might be included among the possible causes of the disease.

Idiopathic Parkinsonism. The terms Parkinson's disease and parkinsonism are used to indicate a syndrome with a characteristic coarse tremor, bradykinesia, and rigidity. Nowadays, at least two forms are distinguished: idiopathic and postencephalitic parkinsonism. Disturbances of the extrapyramidal functions are believed to be common to both forms and particularly the disappearance of dopaminergic impulses from the substantia nigra and the striatum.

Idiopathic parkinsonism (IP) seldom occurs before the age of 40 years, but then the incidence rate increases rapidly with age. The onset of the disease is insidious and the first symptoms are often perceived, both by the patient and by persons close to him (her), as signs of normal aging. IP occurs all over the world, but its incidence varies and is said to be considerably lower among blacks than in whites (*746,747*). Many investigations have claimed that the disease occurs earlier in men than in women, and the prevalence, in age-corrected materials in the age interval 50 to 70 years, showed a male/female ratio of about 1.3 to 1.5 (*747,748*). However, in a more recent investigation, no such difference was observed (*749*).

Patients with IP have a reduced life expectancy, but the disease is generally not regarded to be the cause of death. This often causes the diagnosis of idiopathic parkinsonism to be "forgotten," both in hospital records and in death certificates. This has made epidemiological comparisons difficult over long periods of time (*746*). According to one investigation (*750*), however, there is reason to believe that IP, at the turn of the 19th century (before the epidemic encephalitis around 1920), had approximately the same incidence as now.

Many recent investigations show that patients with IP also have a concurrent SDAT in 30 to 50% of the cases (*751–754*). Conversely, patients with SDAT have, in about 60% of the cases, shown signs of extrapyramidal disturbances of parkinsonism type (*755*).

Neuropathologically, a depletion is seen especially of neurons in the substantia nigra and locus ceruleus (*756,757*), but also of the largest neurons in the striatum (*758*). In principle, the same changes are observed in postencephalitic parkinsonism. More specific for IP is the presence of Lewy bodies in neuronal cytoplasm. These changes are seen in most of the monoaminergic centers in the CNS (*759,760*) and have also been reported, for example, in sympathetic ganglia (*760,761*), nerve endings, and in the adrenal medulla (*762*). The pathogenic mechanism is not understood, and Lewy bodies are said to be fairly heterogeneous (*763*) and to contain filamentous structures antigenically related to normal neurofilaments (*764*). NFD is seen in cases where concurrent symptoms of dementia exist. The remaining neurons in the substantia nigra also show atrophy and reduced nucleolar size as an indication of decreased metabolic activity (*765*).

The ultimate cause of IP is unknown. Viruses have often been suggested as a possible cause, but evidently no clear experimental support exists for this hypothesis (*766*).

FEATURES OF SPECIAL INTEREST: Very few investigations on IP have concerned the occurrence of Al^{3+} in the brain, and those performed are very preliminary. One group has reported increased amounts of Al^{3+} in the arterioles in the globus pallidus (*475,476*). The investigation, performed with an electron probe technique with a fairly low sensitivity, could also detect lead, manganese, and barium. Another group found considerably increased concentrations of Al^{3+} in the brain in two cases of IP (*116*).

It is interesting to note that chronic manganese poisoning produces a picture of parkinsonism and occasionally even dementia (*767,768*). Mn^{2+} has a much more rapid metabolism than Al^{3+} and is easily taken up by the brain. In experiments with $^{54}Mn^{2+}$ in rhesus monkeys, binding to the brain and especially to the basal ganglia and cerebellum appeared very stable (*769*). The mechanism for its toxicity is not known but according to a current hypothesis, Mn^{3+}/Mn^{2+} could catalyze the autooxidation of dopamin, and this would then give rise to cell-toxic substances (*770*).

As in ALS, there might be a connection to peptic ulcers. In a Swedish series of 200 patients with parkinsonism and an equal number of controls, 14% of the patients had a previous history of gastric or duodenal ulcers, verified by X-ray or surgery (*771*). In the controls the incidence was 4%. The mean interval between the onset of gastroduodenal and neurological disease was 14 years (range 1–33), and parkinsonism seemed to appear at a lower age in patients with a history of ulcers than in others. No attempt to interpret the findings was given, nor any mention of whether the ulcers were treated with antacids. Another worker reported no connection between ulcers and IP (*746*).

COMMENTS: The epidemiology and natural history of the disease is in good agreement with the above description of hypothetical diseases caused by Al^{3+} (Table 13). The epidemiological connections to SDAT and ALS (both classic and endemic forms) suggest that IP is a variant, in monoaminergic neurons, of a more general, advanced-age disease in the CNS.

If the present incidence really does not differ from the incidence 100 years ago, this is remarkable and should be considered in any etiological hypothesis (*772*). Probably, very few environmental agents should be expected to have a constant occurrence over a period of time when the common way of life has changed considerably. Al^{3+} might be a possible exception.

Summing up, vague indications exist to associate IP with Al^{3+}; no hard facts seem to argue definitely for or against. The parkinsonism caused by Mn^{2+} is of particular interest and may indicate that several pathogenic agents exist.

Down's Syndrome and Other Forms of Aneuploidy. Down's syndrome (DS) is always induced by trisomy of chromosome 21. In more than 90% of all cases, it appears through a meiotic nondisjunction, most often during meiosis I and mostly in the mother. In the remaining cases various other mechanisms are responsible.

The probability of a pregnancy resulting in a child with some form of aneuploidy increases exponentially with increasing age of the mother. Trisomy and other forms of aneuploidy also occur in the same individual or in the same family considerably more often than would be expected if the occurrence was entirely random. It is believed, therefore, on epidemiological grounds, that age-related causal factors exist (genetic or environmental) for all forms of aneuploidy (*773*). However, no specific environmental factors have been identified. A genetic factor is also suggested by the increased occurrence of DS among more distant relatives to DS individuals (*774*). A 15-fold increase was seen among first-degree relatives (brothers and sisters), an 8-fold increase among second-degree (e.g., aunts), and a 3-fold increase among third-degree (cousins).

DS occurs all over the world, and no significant difference in the incidence rate has been observed. Several studies have attempted to observe any change in the incidence over the last 10 to 20 years. After the data have been corrected for changes in age distribution of the mothers, known abortion frequencies, etc. no significant change in incidence has apparently been observed (*775–780*).

FEATURES OF SPECIAL INTEREST: Some reports have demonstrated an epidemiological connection between DS, on one hand, and SDAT, diabetes, or leukemia, on the other. In all these cases there is also evidence suggesting a double connection, i.e., a correlation both with the tendency for meiotic nondisjunctions and with the DS-phenotype.

Individuals with DS show a very high frequency of SDAT with typical histological changes (*781–785*) and, according to one report, also an increase of Al^{3+} in the

brain (*108*). One group has reported that relatives to patients with SDAT probably have an increased incidence of DS (fivefold in the studied series) (*625–627,786*). Another group, studying a smaller series, found a threefold increase (*628*) but a third group was not able to verify these findings (*787*). The possible double connection between DS and SDAT caused the first group to forward another hypothesis that a defective gene, located on chromosome 21, both predisposes for SDAT and increases the risk of nondisjunction. The group also speculated that the genetic defect might be expressed through a disorganization of microtubules (*626*).

An increased prevalence of diabetes has been reported among younger individuals with DS (*788*) and among individuals with Turner's (45,X) (*789*) and Klinefelter's syndrome (47,XXY) (*790*) and also among relatives to individuals with all these conditions (*791,792*). Therefore, a genetic linkage could possibly exist between the tendency for nondisjunction during meiosis and the tendency to develop diabetes.

People with DS often develop leukemia (the risk is increased by about 20 times) (*793*). Several workers have suggested that an increased incidence of leukemia also exists among the relatives of individuals with DS (*625,794*). Therefore, the tendencies for meiotic nondisjunction and for developing leukemia might also be genetically linked.

DS individuals have an increased susceptibility for infections and are also believed to have a higher risk for autoimmune conditions. In this field a fairly extensive literature has evolved in recent years, but many details are contradictory. However, data concerning some defect in the T-lymphocyte system show fairly good agreement. This defect has mostly been demonstrated by a reduced activation with phytohemagglutinin (PHA) (*795–801*). Some authors have also found reduced numbers of T-lymphocytes (*795*) or have reported PHA reactivity to decrease with increasing age (*802–804*). However, others have reported normal PHA reactivity in DS (*805,806*).

A Russian group has, in several articles, reported structural changes in the lymphocyte chromatin from DS individuals causing a raised melting point for the DNA. The changes were also reported to be present, at a lower frequency, in mothers and siblings of DS individuals (*807*). As yet, these results are unconfirmed by others.

COMMENTS: DS and other forms of aneuploidy show epidemiological features that are in agreement with the above deduced description of Al^{3+}-related diseases (Table 13). Oocytes are also very large cells that do not divide for long periods of time and therefore, theoretically, they could be particularly susceptible to the accumulation of Al^{3+}. No investigations of their Al^{3+} content is known to have been performed.

Apparently, cell biology of meiosis is still not completely understood. The causes of occasionally occurring nondisjunction are almost entirely unknown. However, it is generally assumed that the increased risk for nondisjunction with increasing age is associated either with changes in the chromosomes themselves or in the spindle apparatus (*808*). Possibly, the chromosomes may acquire an increased tendency to stick or to hook on to one another with nondisjunction as a result. In plant experiments, Al^{3+} is reported to have made the chromosomes sticky, produced analphase bridges (*182*) and caused chromosomal breakage and other forms of chromosomal mutations (*183,184*). Therefore, it seems not unreasonable that Al^{3+} might have the same effects on aging oocytes.

The so-called satellite association might be an example of increased chromosomal stickiness. Chromosomes 13, 14, 15, 21, and 22 are "acrocentric" and have a "satellite" connected to its short arm by a constricted part of DNA. During cell division acrocentric chromosomes are occasionally seen together, seemingly connected by their satellites. This phenomenon has been called satellite association (*809*) and has been studied mostly in mitotic lymphocytes but is known to occur also in meiosis. According to several sources, lymphocytic satellite association occurs in a high frequency in DS individuals, according to some reports even in their mothers (*810*), but other workers have not been able to verify these findings. However, it appears improbable that satellite association by itself is a major cause of nondisjunction, as chromosome 16, that lacks this type of satellite, is believed to be the chromosome most commonly involved in nondisjunction (*811*). Instead, satellite association might be a sign of some general change in the chromatin, causing increased stickiness and an increased risk for nondisjunction (*812*). Hypothetically, an increased concentration of Al^{3+} might be such a change.

According to another view, nondisjunction in meiosis I may, instead, be due to an incomplete or missing initial pairing of the homologous chromosomes which might then orientate towards the same centriole (*813*). The tendency for incomplete pairing of chromosomes can be observed as a reduced chiasma frequency and it is said to be a normal finding in oocytes from older individuals. The cause of the incomplete pairing could possibly be an increased condensation of the chromosomes in aged oocytes at the stage when pairing occurs (*814*). Slow accumulation of Al^{3+} might possibly give this effect.

In some cases, nondisjunction is believed to be due to changes in the spindle apparatus. Colchicine interferes with the microtubules and destroys the cell spindle. In high concentrations it causes a complete "C-mitosis" and results in tetraploidy, but in lower doses nondisjunction of occasional chromosomes can instead occur (*547*). Several metal ions (Hg, Pb, and Sn) also induce nondisjunction in meiosis I and are believed to affect the spindle apparatus (*808*). However, the effect has been much stronger for organic compounds, e.g., methyl mercury and ethyl lead, than for inorganic salts, but this may be due only to organic compounds being more easily taken up by the cells. As discussed in the above review, Al^{3+} may have a colchicinelike effect and may inhibit polymerization of tubulin to microtubules. Accumulation in oocytes for several decades might possibly interfere with the normal function of the spindle apparatus, but Al^{3+} should probably have very little effect in short-term studies due to the limited cell uptake and the slow cell metabolism.

Theoretically, Al^{3+} may tend to accumulate in the chromatin particularly of large, long-lived, nondividing cells, and it could have a capacity to interfere with meiotic processes. Then, it might be a possible contributing factor behind nondisjunction during the meiosis; several epidemiological features are also reasonably consistent with such a hypothesis. However, no direct investigations have been performed. Al^{3+} may also be involved in some of the changes characterizing the DS phenotype.

Mitotic Nondisjunctions. Nondisjunctions also occur in mitotic cell divisions and probably often result in nonviable cells. However, in cases when viable cells do originate, single cells or cell clones with aneuploidy may be demonstrated. For technical reasons, aneuploidy has been studied mostly in lymphocytes (often T-lymphocytes). The number of eneuploid lymphocytes increases with increasing age from about 1% to 5–10% and aneuploidy is more common in females than in males (*815–821*). This latter difference is due to the most common, and probably also least harmful, change being the loss of one of the X-chromosomes.

Malignant cell clones are often aneuploid. In many cases, an already malignant clone with a disturbed mitotic mechanism may have given rise to various aneuploid subclones. However, in other cases, the connection is very obvious between a certain type of aneuploidy and the clinical picture; e.g., monosomy 7 in acute myeloid leukemia (*822*), trisomy 12 in chronic lymphatic leukemia (*823–827*) in humans, and trisomy 15 in T-cell lymphoma in mice (*828*). It is therefore assumed that aneuploidy in some way may contribute to the malignant properties of the clone but it has been very difficult to obtain substantial evidence for this assumption.

FEATURES OF SPECIAL INTEREST: Some groups have reported an increased frequency of aneuploidy or chromosomal breakage in lymphocytes from SDAT patients (*829–831*) but other groups have reported no observable change (*820,832–834*).

As stated earlier, reports exist on epidemiological connections between, on the one hand, leukemias and, on the other, SDAT and DS, but these connections cannot, at present, be regarded as being substantiated.

Some epidemiological and clinical observations concerning chronic lymphatic leukemia are also of interest. The disease has a markedly increasing incidence with increasing age and a higher incidence in males than in females. Despite the leukemia generally being due to a monoclonal B-cell proliferation, there are numerous reports on changes occurring also in the T-lymphocytes, e.g., a reduced reactivity to mitogenic substances (*835–837*). In certain ways, the changes appear similar to those in normal aging.

COMMENTS: Al^{3+} appears to have properties that could make it theoretically possible for the ion to cause nondisjunction and aneuploidy by either directly influencing the chromosomes or the microtubules of the mitotic spindle. Whether this actually occurs under normal conditions has evidently not been investigated; probably the possibility has not even been discussed. To the author's knowledge no cytogenetic investigations have been performed in patients exposed to Al^{3+} intoxication by hemodialysis, or in experimental animals that have been subjected to Al^{3+} poisoning.

Difficulties may be encountered in substantiating any possible connection between Al^{3+} and nondisjunction. The amount of Al^{3+} necessary to induce nondisjunction can possibly be detected in an isolated cell by using microprobe techniques. However, the quantity is already halved by the mitosis causing the aneuploidy. The probability of detecting such a cell can be low but greatly increases if additional cell divisions occur after the nondisjunction. However, this might give a negative correlation between the Al^{3+} cell content, and the apparent occurrence of aneuploidy even in a case where the probability of nondisjunction occurring in a mitosis would have correlated positively to the cell content of Al^{3+}. A particular problem may also be that high Al^{3+} amounts in lymphocytes could inhibit the activation by mitogenic substances (*712*) and then prevent any possible aneuploidy being detected.

Though valid positive indications are completely lacking, Al^{3+} cannot be excluded as an important agent in the causation of mitotic nondisjunctions. In its extension, this might have far-reaching implications. In the above review it was concluded that Cr^{3+} probably is the final carcinogenic species, but its slow effects would probably not have been discovered if Cr(VI) had not existed. Considering the many similarities between Al^{3+} and Cr^{3+}, Al^{3+} might then also have carcinogenic properties, though very slow-acting and difficult to demonstrate. However attractive this hypothesis is, it must remain purely speculative as it may be impossible to test it in a satisfactory manner.

Aging of the Cellular Immune Defense System. Many different observations suggest a declining-cellular immune defense activity throughout adult life and particularly in advanced age. Morphologically this is indicated by the involution of the thymus and by a decreasing number of germinal centers in the lymph nodes (*838*). Functionally it may be observed, for example, by a diminished proneness for delayed hypersensitivity reactions and a decreasing tendency to reject transplantates (*839*). Cytologically, the aging is characterized, according to many reports, by a reduction of the number of circulating T-lymphocytes and of their ability to be activated by various mitogenic substances (*838–842*). Apparently, no obvious reason for the aging of the immune defence system has been proposed.

FEATURES OF SPECIAL INTEREST: The immunologic changes occurring in chronic renal failure and hemodialysis are characterized by increasing anergy to skin tests, a decreasing tendency to host versus graft reactions and delayed hypersensitivity and an increasing occurrence of autoimmune phenomena (*843*). The most significant changes are said to be a low number of T-cells and their nonreactivity to PHA and other mitogenic substances. The primary cause for these changes is unknown. However, it is interesting to note that plasma from patients with impaired renal functions is said to inhibit the PHA-reactivity in lymphocytes from healthy indi-

viduals. Conversely, lymphocytes from patients, after careful washing and resuspension in plasma from healthy persons, show normal PHA reactivity. Therefore, it has been proposed that the T-lymphocytes are essentially normal but are exposed to a harmful factor in the plasma causing various functional disturbances and a reduced life-span. However, as implied in the discussion above, the changes observed in hemodialysis patients also show many similarities to the changes associated with normal aging.

It is also of interest to note that many patients with endemic ALS-PD from Guam are reported to show signs of reduced cellular immunity in skin tests, a reduced number of T-lymphocytes and also, in many cases, a reduced PHA-reactivity (*697*). Essentially the same findings are reported in IP (*844*) and similar changes also occur in DS.

COMMENTS: The lymphocytes of lymphoid organs and other tissues are fairly large, metabolically and immunologically active in various ways, and have a relatively limited life-span. The T-lymphocytes circulating in the blood are mostly small dormant cells, memory cells, that are believed to survive for long periods (possibly decades). A lot of evidence indicates that lymphocytes have a particularly high affinity for Al^{3+} and the Al^{3+}-like ions. Probably, large active cells have the highest affinity, but small cells may compensate the lower affinity by having a considerably longer life-span. No investigation concerning the concentration of Al^{3+} in lymphocytes is known to have been published, and if an investigation was performed, no increase (in the average Al^{3+} concentration) with increasing age would probably be found. Elimination of the oldest cells, most likely containing the highest Al^{3+} contents, and production of new cells with low Al^{3+} contents should maintain a steady state within the lymphocyte population. At the same time, a considerable and possibly even toxic cellular accumulation might occur in the individual lymphocytes.

Some observations suggest that the immune system, and particularly the T-lymphocytes, may be sensitive to the toxic effects of Al^{3+}-like ions (*256,260,327,457*). The changes in the cellular immune defense found with normal aging, and in DES, endemic ALS, IP, and DS are also in good agreement with experimentally observed Al^{3+}-effects (*712*) and with effects considered possible in relation to the ion's biochemistry and metabolism.

Summing up, the biochemistry and the metabolic behavior of Al^{3+} suggest that it might be involved in T-lymphocyte aging but so far no experimental investigations have been performed.

Hypersensitivity and Autoimmunity. Metal cations often induce hypersensitivity. However, free ions are far too small to be immunogenic by themselves or able to trigger allergic symptoms (apart from the fact that true free ions of the current elements are scarcely found in the organisms). A metal-ion hypersensitivity, therefore, always involves a complex of the ion (hapten) and a larger molecule (carrier), usually a protein. Cations with a high charge/radius ratio can easily alter the conformation of proteins to which they are bound. This can expose new antigenic determinants and induce sensitization. However, the ion that alters the conformation need not, in principle, be an integral part of the new determinant. Hypersensitivity to metal ions shows, therefore, a comparatively low specificity for the cation—and may then have a tendency for cross-reaction with similar ions (*845*)—but a high specificity for the carrier, the denatured autologous protein. For this reason, metal allergies have occasionally been compared with autoimmune conditions (*845–848*).

The formation of complexes between Al^{3+}-like metal ions and their carriers should occur according to the earlier discussed model of how the ions may behave in the organisms. The various binding sites on extracellular proteins should, in principle, be occupied in a particular order governed by the affinities of the ligands and the stability of the complexes. If the concentration of the ion is very low, only binding sites having the highest affinity and stability can be occupied. If the complexes then formed happen not to be allergenic, then, no hypersensitivity can probably be induced. This may explain why Fe^{3+}, bound to transferrin to an extremely high extent, very seldom causes hypersensitivity. If the concentrations of free ions and low molecular weight complexes are locally increased somewhere in the body, then new complexes may be formed and sensitization could occur. However, if concentrations increase more generally in the body, immunologic tolerance usually arises. Thus, hypersensitivity is easily induced if Be^{2+} is experimentally administered intratracheally or intradermally to guinea pigs, but if Be^{2+} is then given intravenously or orally, the animals develop tolerance (*344*).

FEATURES OF SPECIAL INTEREST: Cr^{3+}, Be^{2+} and Zr^{4+} are all well known for their proneness to cause hypersensitivity, while Al^{3+} is generally regarded almost never to do that. Hypersensitivity to the other Al^{3+}-like ions is similarly almost unknown. The sensitizing potentials of Cr^{3+}, Be^{2+}, and Zr^{4+} are, however, moderate when compared to other allergens. Therefore, it is possible that the reason for hypersensitivity to Ga^{3+}, In^{3+}, Sc^{3+}, and Y^{3+} not being known is because of their scarcity in the environment. One investigation, however, showed In^{3+} to be strongly sensitizing in guinea pigs (*849*).

Hypersensitivity induced by metal cations usually is of the delayed, cell-mediated type (type IV), that clinically causes eczema, e.g., contact dermatitis to Cr^{3+}, or allergic granulomatosis, as for Be^{2+} and Zr^{4+}. However, granulomas may also arise as a foreign-body reaction without any immunological mechanisms being involved (*358,850*). Al^{3+} hydroxide is known to cause this type of granulomas (*356,360,851–853*), as are poorly soluble salts of Be^{2+} (*358*) and Zr^{4+} (*353,354*) when administered to nonsensitized individuals. The distinction between allergic and toxic granulomas is not very certain.

In experimental immunization and in vaccination, Al^{3+} (as hydroxide, phosphate or directly as antigen complexes) has been used extensively as an immunologic adjuvant, i.e., a substance that potentiates the specific immunization (*854,855*). In animal experiments, Be^{2+} has

been shown to have a similar effect (*856–858*). Plausible mechanisms causing the effect may either be direct interaction with the antigen (exposing new determinants or causing a more protracted antigen release from the injection site) or some effect on the participating cells (the production of foreign-body granulomas might favor the immunization against the specific protein antigen) (*855,859*).

COMMENTS: Due to its common occurrence, its close chemical similarities to Cr^{3+}, Be^{2+}, and Zr^{4+} and its effect as an immunologic adjuvant, Al^{3+} would also be expected to induce hypersensitivity. This, however, is very rare (*860–862*), and this is evidently something that has never been explained. Yet, several reasons for the rarity are conceivable.

A possible reason why skin exposure to Al^{3+} does not induce contact dermatitis may be that the ion has difficulty penetrating the skin due to its low solubility at neutral pH and its great tendency to bind to the outer skin layer (*863*) where no immunologic activity occurs. Cr^{3+} has the same difficulty, and exposure to Cr^{3+} seldom results in sensitization or symptoms in an individual already sensitized. Highly soluble Cr(VI) compounds, however, easily enter the skin but are later reduced to Cr^{3+} and then cause allergic reactions (*428*). Zr^{4+}, too, has difficulty in producing hypersensitivity on application to intact skin; prolonged exposure and high concentrations are necessary, and even then only a few individuals become sensitized. Therefore, among the ions discussed here, Be^{2+} has the greatest tendency to induce hypersensitivity by direct skin contact. As for Cr(III)/Cr(VI), however, special mechanisms may exist for both Zr^{4+} and Be^{2+}, as opposed to Al^{3+}, that help them penetrate the skin and induce the hypersensitivity (see note 21).

Another important reason why Al^{3+} does not induce allergy may be the larger amounts of Al^{3+} present in the body, compared to Cr^{3+}, Be^{2+}, and Zr^{4+}, that might normally cause an immunologic tolerance to the Al^{3+}-induced conformation variants of the body proteins. Be^{2+} and possibly also Cr^{3+} administered orally or parenterally to guinea pigs have produced tolerance (*344,358,864*), but no similar reactions seem to have been observed in humans. A possible tolerance to Al^{3+} should not, however, concern the ion *per se* but apply to specific Al^{3+}–protein complexes. If the concentration was to increase locally, new complexes would be formed, according to the discussion above, and some of these should, probably, be allergenic.

A third explanation for Al^{3+} not inducing hypersensitivity may possibly be that Al^{3+} could exhibit a toxic effect directly on the T-lymphocytes (see above), which could impair all cellular immunity reactions. Be^{2+} and Ga^{3+} are reported to show such toxic effects (*256,327,457*) but, evidently, this has not made Be^{2+} hypersensitivity impossible. This explanation may, therefore, seem less valid.

A fourth explanation—and certainly a very speculative one—as to why Al^{3+} hypersensitivity has not been observed might be that such conditions do exist but their allergenic connections to Al^{3+} have been overlooked. Allergies show very varied dose–response relations and Al^{3+} compounds are abundant everywhere; therefore a possible association between Al^{3+} and certain clinical, allergic conditions would be difficult to establish epidemiologically. A person who developed a hypothetical Al^{3+} hypersensitivity need probably not show any increased Al^{3+} concentration in his tissues or urine. Only a few percent of workers that in the past were industrially exposed to Be^{2+} actually developed berylliosis, and these individuals had no increased Be^{2+} concentration in their tissues or urine compared to other similarly exposed workers (*847*).

Table 14. Possible properties of a hypothetical hypersensitivity induced by Al^{3+}. The properties have mainly been derived from the metabolism of Al^{3+} and from common properties of other metal ion hypersensitivities.

Worldwide occurrence with a fairly constant incidence over time
Possible correlation, in large series, to Al^{3+}-minerals and lack of strict dose-dependency in individual cases, as generally is the case in hypersensitivities
Possible hereditary disposition and incidence differences between races
Onset most common before middle-age, as in other hypersensitivities
Insidious onset, compared to other hypersensitivities, and a chronic course due to the slow metabolism of Al^{3+}
Changes occurring mostly in organs with high Al^{3+} concentrations, e.g., lungs, skin, and lymph nodes
Histologic changes possibly localized mostly to connective tissue, basement membranes, cell nuclei and chromatin, as these structures particularly accumulate Al^{3+}
Symptoms mostly from the lungs, as in Be^{2+} hypersensitivity and in toxic (nonallergic) Al^{3+} reactions
Cell mediated, delayed hypersensitivity mainly involving the T-lymphocytes, as for other metal ion hypersensitivities
Granulomatosis as in Be^{2+} and Zr^{4+} hypersensitivities and in Al^{3+} foreign-body reactions

If the above employed method (Table 13) of deducing from the biochemistry and metabolism of Al^{3+} is also applied to describe the probable characteristics of a hypothetical Al^{3+} hypersensitivity, then the description proposed in Table 14 is possible. It is obvious that this description, in many respects, conforms very well with current concepts of sarcoidosis and this disease is discussed in a separate section below. If Al^{3+} did induce hypersensitivity, but no connection to Al^{3+} was recognized, then this undetected hypersensitivity could easily have been classified as an autoimmune condition. Experimental autoimmune conditions have been induced in tissues by injecting immunologic adjuvants or mixtures of these and autologous proteins. Usually Freund's complete adjuvant has been utilized, and few reports are known of Al^{3+} being used; one reported, however, that alum-treated rabbit thyroid extracts injected to rabbits caused high titers of thyroid autoantibodies in two-thirds of the animals, but no clinical thyroiditis was observed (*866*). It may also be of interest that berylliosis has occasionally been characterized as an autoimmune disease

(*846,847,867,868*), and in berylliosis autoantibodies to various tissues and even DNA have been detected (*867*).

It has often been proposed that unknown antigens or haptens could possibly cause autoimmune diseases. Recently, a theory has been forwarded, suggesting that all drug-induced lupus erythematosus may be regarded as an "adjuvant disease" (*869*). Al^{3+} is probably quite unique, among well-established immunologic adjuvants, in being normally present in the tissues. The concentration found in structures where Al^{3+} is accumulated is probably no less than the drug concentrations that induce lupus erythematosus. The affinity to collagen and the proneness of Al^{3+} to accumulate, for example, in the skin and the basement membranes of renal glomeruli and small blood vessels could also localize the hypothetical process in a way that closely agrees with the changes seen in systemic lupus erythematosus. If a life-long accumulation of Al^{3+} occurs in certain structures, then the probability of hypersensitivity or autoimmunity developing should also increase with age, as has been observed in several autoimmune diseases.

Summing up, the nonexistence of hypersensitivity to Al^{3+} is unexplained and very remarkable. Speculatively, its very sluggish metabolism might cause clinical pictures that have neither been associated with Al^{3+} nor with hypersensitivity.

Sarcoidosis. Sarcoidosis is characterized by granulomatous, noncaseating changes most commonly observed in the lungs and hilus lymph nodes. It is also commonly found in the skin, eyes, liver, spleen, lymph system, and skeleton, and the disease may otherwise occur in practically all organs. The diagnosis can be established by an immunologic reaction, Kveim's test. The fact that one and the same reagent appears to detect the disease in all parts of the world has been taken as an indication that sarcoidosis is a true etiologic entity (*870*). Usually, the disease occurs in a more acute form, which is believed to heal and disappear spontaneously within 1 to 2 years. This form causes fairly few symptoms, and most observed cases have been found more or less accidentally on lung examinations. Therefore, many mild cases may also go undetected. Acute lung sarcoidosis is characterized by enlarged hilus lymph nodes with a typical histological picture. More chronic forms have a poorer prognosis and are primarily characterized by a granulomatous fibrosis of the lung parenchyma with less apparent hilus node enlargement. The disease is considered rarely to be fatal, and Kveim's test is reported to be positive in 80 to 90% of acute cases but in less than 50% of chronic cases.

Sarcoidosis is represented all over the world, but the reported incidence varies considerably, probably due in part to diagnostic differences. The disease is mostly observed between the ages of 20 and 40 and, according to several reports, it has a somewhat higher incidence in women. Blacks are reported to have a roughly 10-fold higher incidence than whites. In several reports, the incidence is stated to be higher in rural areas than in large cities, and among U.S. blacks roughly a 10-fold increase is reported in farm workers compared to city residents (*871*). In the U.S., a geographic difference of incidence has also been observed, suggesting, according to some authors, the existence of important geologic or ecologic factors.

Despite the fact that the disease has been known for about 100 years, its etiology is still unknown. A genetic factor is believed to exist (*872*). Earlier etiologic hypotheses have mainly concerned various microorganisms (mycobacteria, fungi and viruses). Pine pollen has also been discussed (based on the geographic distribution of pine forests in the U.S.) and autoimmunity (*873*).

The disease is characterized by considerable changes in the immunologic system. Cellular immune defense activity is clearly reduced. In circulating blood the T-lymphocytes are reduced in number, and they show a poor reactivity to phytohemagglutinin, PHA. However, the B-lymphocytes are often increased as well as the concentration of immunoglobulins. Large numbers of activated T-lymphocytes are present in disease foci, i.e., cells showing signs of metabolic activity and, probably, also dividing. The essential immunologic change is believed to be an activation of the T-cells causing them to stop circulating in the blood and instead to accumulate in the disease foci and in the lymph system (*874–876*). The reason for the activation and for the tendency of the condition to persist is not known, but according to a current hypothesis some chemical or microbiological agent might affect the T-cells and possibly modify some of their cell surface antigens. This would result in the T-cell activation and the development of granulomas (*870–872*). This agent could have properties that prevent it from being destroyed or, by other means, help it persist in the changes, whereby the process could be chronic.

FEATURES OF SPECIAL INTEREST: Ga^{3+} is accumulated very distinctly in active sarcoidosis foci, and $^{67}Ga^{3+}$ scanning has become one of the most important methods for localizing the changes and following their activity.

Inclusion bodies occur in epithelioid cells and giant cells, and many of them apparently consist of electron-dense residual bodies from lysosomes containing, among other things, calcium and iron (*870*).

Granulomas associated with sarcoidosis are histologically very similar to those seen in Be^{2+} and Zr^{4+} hypersensitivity (*877–879*); also clinically, berylliosis and sarcoidosis are so similar that the true diagnosis is often dependent entirely on a possible anamnestic history of Be exposition or the result of skin testing with Kveim's extract or Be^{2+} respectively (*880*). Similarly, Ti^{4+} is also reported to cause pulmonary granulomatosis, which histopathologically was taken for sarcoidosis (*881*).

COMMENTS: Much of the histological picture and the organ localization is quite consistent with the hypothesis that Al^{3+} causes or contributes to sarcoidosis. The fact that Ga^{3+} is readily taken up by sarcoidosis foci probably implies that Al^{3+} is also taken up. The epidemiology of sarcoidosis is also consistent with the hypothesis of Al^{3+} functioning as an etiologic agent. Al^{3+} belongs to a rather limited group of substances that are indeed found throughout the world. Increased exposure in rural areas

and in farm work appears reasonable, as does the possible association to special geological zones.

Mineral particles are taken up by the lungs and hilus lymph nodes and may slowly release Al^{3+}. Normally small foreign-body granulomas may appear but no symptoms. In genetically disposed individuals this process might proceed to an allergic reaction. T-lymphocytes become activated, and the granulomatous tissue reaction becomes more pronounced and widespread and might even extend to other organs. The changes take up and bind Al^{3+} from the blood, probably through the action of the macrophages and activated lymphocytes. The continual uptake of Al^{3+} in the affected tissue might make the process, once initiated, self-sustaining, but it might be terminated by immunologic tolerance developing for the hypothetical Al^{3+} antigen.

Only certain specific compounds with "medium" solubility probably induce the disease. More easily soluble compounds might give rise only to diffuse lung fibrosis (*853,882,883*) and more insoluble compounds might possibly be inactive. Epidemiological studies on people industrially exposed have not revealed any increased occurrence of sarcoidosis, but some cases of lung fibrosis have been reported. Other routes of administration, e.g., dialysis, might induce immunologic tolerance or decrease the ability for this type of reactions (see above).

Chemically, Al^{3+} is similar to Be^{2+} and Zr^{4+} and, in respects where the ions differ, Al^{3+} is often positioned intermediately between the other two ions (see review above). All three produce similar foreign-body granulomas. Any possible Al^{3+} hypersensitivity should, therefore, be expected also to induce granulomatosis. The uptake of Al^{3+} in the granulomas might be nonspecific and might have no connection with Al^{3+} (according to the hypothesis) being the ultimate cause of the process. Al^{3+} may be taken up by all granulomatous tissues. Al^{3+} could function as a hapten in a protein complex or as an immunologic adjuvant with a different mode of action.

Similarities between the changes in sarcoidosis and Be^{2+} and Zr^{4+} granulomatosis led to an investigation in which healthy individuals and sarcoidosis patients were tested intracutaneously with ions from 71 different elements (*878*). Al^{3+} chloride was included in the investigation. However, none of the ions showed any reaction whatsoever. This probably does not exclude the possibility of Al^{3+} being involved. The test amounts injected were small, only 2 μg, but it is not clear if this quantity referred to the complete salt, $AlCl_3 \cdot 6H_2O$, or just the Al^{3+} ion. Therefore, the amount injected was equivalent to the quantity of Al^{3+} normally found in 0.2 or 1.5 g of skin (*21*). Likewise, for Be^{2+}, it is assumed that a negative skin test does not exclude berylliosis (*884*). Possibly, the ions must be administered as a complex with the specific carrier for the testing to be successful.

The possibility that Al^{3+} may cause or contribute to sarcoidosis is not known to have been discussed previously. The present hypothesis is based on pieces of evidence, which all appear plausible in relation to the biochemistry and metabolism of Al^{3+}, when viewed separately but, of course, the entire hypothesis is very speculative.

Maturity-Onset Diabetes. Maturity-onset diabetes (MOD) (also termed non insulin-dependent diabetes, NIDD) is characterized by a reduced glucose tolerance, probably caused by reduced insulin sensitivity (insulin resistance) in the tissues. Early in the disease glucose-induced insulin secretion is normal or even increased but later it may decrease.

MOD may be found already at the age of 20, but the incidence increases later and is roughly doubled every 10 years up to 60 years of age. The onset is always insidious—contrary to juvenile diabetes—and is believed to be preceded by a prediabetic stage often lasting many years. This has caused great trouble for epidemiological investigations since the diagnosis has become highly dependent on the results of laboratory tests (fasting blood glucose and glucose tolerance tests), even though there has been a lack both of standardized criteria for the diagnosis and of instructions as to how the laboratory tests should be performed.

MOD apparently occurs throughout the world, but its reported incidence and prevalence varies considerably, possibly partly due to diagnostic differences. MOD may appear endemically with very high prevalence, occasionally more than 50% in adults. This applies especially to some populations that have recently adopted a Western lifestyle, causing an earlier latent disposition for diabetes to become manifest. The best known examples are several native American tribes (e.g., Pimas) (*885*) and certain Micronesian groups (e.g., on Nauru) (*886*). According to reports, endemic MOD also occurs on Guam (*887,888*). The occurrence of endemic MOD suggests that environmental factors are of etiological importance.

Being overweight has been shown to be an important risk factor for MOD, and real obesity is believed very often to cause the disease even in the absence of other etiological factors (*889*). Obesity is also believed to be a main cause of the above-mentioned endemic forms.

Contradictory reports exist regarding any possible difference between the sexes. Older reports have, as a rule, shown a distinctly higher incidence in females, but the difference is generally attributed to women being more often overweight than men. More recent investigations have even suggested a higher incidence for men (*890,891*). Commonly used methods in population investigations may also have had a distinct tendency to overestimate the female frequency. For instance, in the glucose tolerance test, the same dose has generally been recommended for all persons, despite the fact that women generally have a considerably lower distribution volume for glucose than men (*889*). It has also been suggested that an insufficient correction for overweight has been allowed for in females, as the reference weight used should in fact represent some degree of overweight compared to the male reference (*889*).

Heredity is believed to be of great importance. Identical twins have shown up to 95% concordance, but part of this can possibly be explained by concordance for body weights (*889*). The inheritance is assumed to be polygenetic, and first-degree relatives of an individual with

MOD are believed to have a 2- to 3-fold increased risk of having the disease themselves. Occasionally, the onset of MOD occurs very early, even before 10 years of age. These cases, referred to as MODY (maturity-onset diabetes of young people), are said to have an autosomal dominant inheritance (*892*). However, the onset of the disease in most family members occurs between 20 and 50 years of age and the earliest cases are often symptomless. The condition indicates that MOD is not just one etiological entity; several types may exist.

FEATURES OF SPECIAL INTEREST: Most patients suffering from advanced renal failure develop a glucose intolerance but, at the same time have an increased insulin release in glucose tolerance tests (*893*). The condition has been referred to as uremic pseudo-diabetes and is believed to be due to peripheral insulin resistance (*894*) but the finer details of the mechanism have not been revealed.

Patients with idiopathic parkinsonism very often (> 50%) appear to have a reduced glucose tolerance and also often develop manifest MOD (*895,896*). Furthermore, an increased frequency of clinical and subclinical diabetes—with a marked insulin resistance (*897*)—is reported in ALS, both of the classic type (*736,898*) and of the Guam type (*899,900*).

As stated earlier, an increased frequency of diabetes is also reported in individuals with Down's, Klinefelter's, and Turner's syndrome and in their relatives (*788–792,901*). Many cases of diabetes in DS individuals are found in the age group below 10 years but the disease has shown, nevertheless, many similarities with MOD.

Idiopathic hemochromatosis (IH) is accompanied in 60 to 70% of the cases by manifest diabetes, and a further 10 to 20% have subclinical diabetes. The disease shows MOD characteristics with signs of insulin resistance. Earlier, the diabetes was thought to be due to the damage of the β-cells by iron deposits, but poor correlation between the amount of these deposits and the occurrence of diabetes (that is often one of the first symptoms of the disease) contradicts this simple interpretation. Another argument against it is the fact that at least 25% of all patients with combined IH and diabetes are reported to have first-degree relatives (parents, children, siblings) with clinically normal MOD without IH (*902–905*). Relatives of IH patients without diabetes showed no such increased frequency. The incidence of MOD, therefore, appears to be equally increased in the relatives of patients with IH diabetes and in those of patients with common MOD. However, certain indications exist that Fe^{3+} deposits could contribute to the diabetes, as the disease also occurs in hemochromatosis induced by Fe^{3+} overloading through blood transfusions (*906*).

COMMENTS: Many features in MOD clearly agree with the above description of hypothetical Al^{3+}-related diseases (Table 13). The epidemiological connections to several of the previously discussed diseases are of particular interest. Like these, MOD has occasionally been described as a form of accelerated aging (*907*). Possibly, therefore, some primary aging mechanism could contribute to induce all these conditions. An interesting, possible connection to aging is the thickening of blood vessel basement membranes, occurring in all forms of diabetes and also in normal aging. Basement membrane collagen has an extremely slow turnover. It is apparently unknown whether the thickening is due to an increased synthesis or a reduced degradation of basement membrane or a combination of both (*908*). Al^{3+} is reported to accumulate in vascular basement membranes and might, theoretically, stabilize it against the normal degradation (see discussion above). Al^{3+} could thereby contribute to the thickening.

The apparently very close connection between MOD and IH is difficult to understand without assuming the presence of some important metabolic property controlling both conditions, and not merely linkage of functionally unrelated genes. In IH, one obvious, abnormal property exists: the tendency to absorb excessive quantities of Fe^{3+}. IH is inherited as a single autosomal recessive trait (*909–911*) and, therefore, it is close at hand to assume that this very property (the only abnormality in IH?) is the common disposing factor behind IH and the diabetes found in the relatives (the IH heterozygotes?). Heterozygosity for the IH allele, therefore, might be one of the genetic background factors to MOD, a hypothesis which has also been forwarded previously (*912*). The heterozygote frequency in Western populations is roughly 10% (*913*). Heterozygous individuals do not develop clinical hemochromatosis but can be shown to have a somewhat increased Fe uptake by laboratory tests.

If one of the inherited properties, predisposing for MOD, were an increased tendency to absorb and accumulate Fe^{3+}, then the same tendency might also apply to Al^{3+}. Apparently, however, no investigation has been performed of the Al^{3+} metabolism in MOD. Accumulation of Fe^{3+} should also be expected to make the Al^{3+} metabolism more rapid by Fe^{3+} displacing Al^{3+} from many stable complexes. Hypothetically, this could result in a reduced intracellular binding of Al^{3+} in the intestinal mucosa and thereby an increased uptake of the ion. The body distribution of Al^{3+} might also be affected by Fe^{3+} (compare discussion on DES).

The alleged connection of Cr^{3+} to insulin, glucose metabolism, and diabetes, discussed above, and the great metabolic and chemical similarities between Cr^{3+} and Al^{3+} may seem to suggest a simple mechanism whereby Al^{3+} might possibly help induce diabetes. However, in no way has this alleged function become generally accepted. Recent literature on insulin receptors and insulin function scarcely even mention Cr^{3+}. Many authors have also reported directly contradictory findings. One group induced, with Cr^{3+} *in vivo*, a blood glucose increase in rats (single IP administration) (*914*) and *in vitro* a reduced insulin release from isolated pancreas islands (*915*). According to another group, Cr labeling of isolated β-cells blocked their glucose-induced insulin release (*916*).

Several investigations have reported an increased intestinal Cr^{3+} uptake in diabetics. Generally, the findings have been interpreted as a compensatory reaction to an assumed Cr^{3+}-deficiency. However, the findings could just as well denote a primary increase.

The cells of the skeletal muscles are, in principle, as old as the individual. Muscle cells, therefore, could accumulate Al^{3+} in spite of their uptake being slower than for many other cells. If the heterochromatin eventually became saturated, then the Al^{3+} concentration could increase in the cytosol and inhibit hexokinase (see above). Hexokinase phosphorylates all glucose molecules used by the cells, as that constitutes the first, rate-limiting step to both glycolysis and glycogen storage. Phosphorylation also causes the transfer of glucose into the cells to become irreversible as glucose-6-phosphate ions cannot cross the cell membrane. Then, hypothetically, inhibition of cytosolic hexokinase by Al^{3+} could interfere with the muscle cell uptake of glucose. Since the main part of the glucose absorbed after a large meal is normally taken up by the skeletal muscles, this could result in a slow whole body cell uptake and an increased extracellular glucose concentration. The insulin release would then increase, and a clinical picture with glucose intolerance and insulin resistance could arise that, in many ways, showed similarities to early MOD or uremic pseudodiabetes. If, in the proposed state, the tissue activity of hexokinase were measured, it could nevertheless be shown to be normal due to addition, in the assay, of excess of Mg^{2+}-ATP which might kinetically displace Al^{3+}-ATP (see discussion above). Recent recommendations to use citrate in the assay to suppress the inhibitory effect of Al^{3+} impurities in commercial ATP preparations (*147*) could, of course, give the same result.

Summing up, several aspects of MOD are easily fitted into the present hypothesis of Al^{3+} contributing to the disease, but no experimental evidence exists, and very few investigations have been performed.

Muscular Dystrophies. Several different forms of inherited muscular dystrophy and myotonia exist, but the following discussion is limited to the two most common forms, Duchenne's muscular dystrophy (DMD) and myotonic dystrophy (MyD) (synonym: myotonia atrophica).

DMD has a recessive X-chromosomal inheritance and therefore affects only boys. The symptoms are observed at 2 to 3 years of age and the condition slowly progresses. The afflicted is usually confined to a wheelchair by the age of 10 years and dies as a result of the disease in his late teens. Becker's muscular dystrophy is a variant with onset in the teens and a considerably slower progress. By the X-chromosome inactivation, females carrying the DMD allele are not truly heterozygous but show a mosaic of normal and affected cells. In gene carriers, changes can usually be detected but clinical symptoms rarely occur. As the DMD allele is a lethal gene in boys, it would rapidly have been eliminated from the gene pool if a high incidence of mutations had not existed—approximately 1/3 of all cases are assumed to be due to new mutations (*917*). MyD shows an autosomal dominant inheritance and disease onset is often between 15 and 40 years of age.

The exact pathogenic mechanisms causing the muscular dystrophies are unknown, even though several of the conditions have been known for 100 years or more. In recent years, a large number of reports have been published concerning abnormalities in cell membranes from erythrocytes, muscle cells, or other cells from muscular dystrophy patients, particularly DMD, and from healthy gene carriers. Most often, other groups have then failed to verify these findings. Therefore, it is impossible to point to any definite changes, and even more impossible to state which of the changes are primary and which are secondary. Despite disagreement existing in many respects (that may be due to a poor definition of the conditions, to the presence of several different but clinically similar mutations or to differences in the techniques used), some agreement is, nevertheless, accumulating on the very existence, at least in DMD, of some membrane abnormality in erythrocytes and muscle cells (possibly in all tissues) that might induce the disease (*917,918*).

Increases or decreases in certain enzyme activities is reported in several muscular dystrophies, both in cells and the circulating blood plasma. The changes are probably secondary to other changes in the cells and not directly inherited. Neither is there any good reason at present to assume that the observed enzyme changes contribute to the pathogenesis.

FEATURES OF SPECIAL INTEREST: A phenomenon very much discussed in recent years in connection with muscular dystrophy is the so-called lymphocyte capping. If lymphocyte membrane proteins (e.g., immunoglobulins and other receptors) are crosslinked by some reagent (e.g., specific antibodies), within a few minutes they become collected to a "cap" over one pole of the lymphocyte and are then rapidly internalized by endocytosis. This phenomenon is believed to be an effect of a continual recycling of the lipid membrane by exocytosis occurring at one pole of the cell and endocytosis at the other. The rapid lateral diffusion normally prevents most membrane proteins from taking part in this process but cross linkage interferes with the diffusion and, therefore, causes capping. Several articles report the capping tendency to be reduced in the lymphocytes from individuals with DMD or from healthy carriers of the gene (*919–923*). Thus, despite also some negative reports, it appears most probable that some change exists. Exactly what may cause reduced capping is not known but a reduced membrane fluidity or an impaired microtubular function have been suggested. Reduced lymphocyte capping has, moreover, been observed in the normal elderly and in individuals with Down's syndrome (*924,925*). However, reduced lymphocyte capping has also been induced by the action of Al^{3+} or Be^{2+} (*921*); its mechanism is unknown, but it is interesting to note that Al^{3+} is reported to interfere both with membrane fluidity and microtubular functions (see discussion above).

Several connections to metal ions—mostly Ca^{2+}—also exist. Thus, it is reported that many muscle fibers from individuals with DMD show a greatly increased stainability with various histological staining techniques. This is particularly true for certain metal-ion chelating stains, e.g., Alizarin Red S and pentahydroxyflavone (Morin). The results were interpreted as evidence of increased Ca^{2+} in the cells, which was believed to be a link in the pathogenesis (*926,927*). The staining methods are, however, extremely nonspecific and are also recommended for

the detection of Al^{3+} (*928*). One group has also detected increased amounts of Ca^{2+} in muscle cells from DMD individuals using X-ray fluorescence (*929–931*). If an increased Ca^{2+} concentration does exist, then it seems very likely that it could be an important but a fairly late step in the chain of events leading to dystrophy.

One of the groups that reported on the increased amounts of Ca^{2+} in muscle cells has also recently reported on the possible existence of a circulating plasma factor in DMD (*932*). The erythrocyte $Na^+ + K^+$-ATPase activity is reduced in DMD. Normally, ouabain has an inhibitory effect on this enzyme. Several workers have, however, reported ouabain to have an activating effect on the remaining low $Na^+ + K^+$-ATPase activity in DMD individuals. The investigation referred to maintains that this is due to the presence of a plasma inhibitor and also states that the same inhibition is observed with erythrocyte membranes from healthy persons after incubation in plasma from DMD individuals. The authors speculate, on the basis of other experiments, that the factor is a metal-ion protein complex but state that Ca^{2+} and Mg^{2+} are probably not involved.

Patients with various forms of muscular dystrophy, including DMD and MyD, are reported to accumulate $^{67}Ga^{3+}$ in their muscles (*933,934*); for DMD this was also observed in healthy gene carriers. If patients with muscular dystrophy also take up Al^{3+} in their muscles, then this may well explain some of the above related findings and could possibly be an important step in the pathogenesis. However, no direct investigation has been performed on the occurrence of Al^{3+} in muscles nor on which cells or structures are responsible for the accumulation of Ga^{3+}. The possibility exists, therefore, that infiltrating connective tissue or lymphocytes are responsible.

Diabetes very often occurs in MyD and is believed to be due to a reduced insulin sensitivity in the tissues. Even in MyD patients without clinical signs of diabetes, the number of insulin receptors in monocytes is reduced considerably. The same change, less pronounced however, has been reported in DM (*935*).

A moderate mental retardation is generally seen in DMD (*917*), and in MyD intellectual changes are normal symptoms, often ending in severe dementia (*917*).

Several cases of MyD in individuals with Klinefelter's and Down's syndrome have been reported, and a possible connection or genetic linkage between these conditions has been proposed (*936*). Cases of concurrent DMD and Turner's syndrome have also reported, but probably can have a natural explanation in the disease's X-chromosomal inheritance (*917*).

As a remarkable coincidence (?), MyD occurs with a high incidence rate on Guam, and families suffering from both ALS and MyD have been observed on the Kii Peninsula in Japan (*937*). Several immunologic abnormalities have been reported in MyD, e.g., a reduced tendency for delayed cell-mediated hypersensitivity reactions (*938*).

COMMENTS: It is obvious that muscular dystrophies have a simple genetic cause. The molecular mechanisms are unknown, but possibly include cell membrane changes. However, it is also obvious that muscular dystrophies, even DMD, have features related to aging. Despite the fact that the disease-inducing gene must be present from birth (and even before), the symptoms of DMD do not appear during the first years of life. When they do appear they are insidious and progress slowly for several years. Therefore, the pathogenesis should include some change, requiring a considerable period of time to develop. This change can hardly be directly localized to the cell membrane as this has a rapid turnover. Possibly, this slow change might affect DNA and could either be due to a primary membrane change or cause secondary membrane changes. If an altered DNA function is part of the pathogenesis, this could easily explain the occurrence of the numerous changes so far reported. Many of the changes could have no direct causal connection to the actual dystrophy but could be pure epiphenomena.

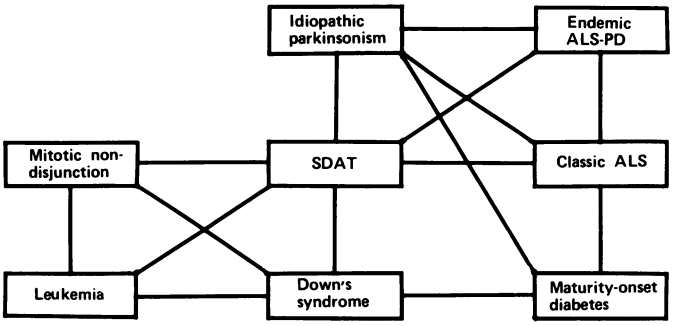

FIGURE 5. Epidemiological connections between some aging-related diseases. The connections are discussed under the respective disease sections.

Applying this interpretation, it may be possible that accumulation of Al^{3+} in chromatin could be the time-dependent step of the pathogenesis. Some form of primary membrane change—different in the various forms of dystrophy—might be the reason for increased amounts of Al^{3+} and Ga^{3+} being taken up by the cells. A somewhat different mechanism for the delay and for the cellular effect is also conceivable. Accumulation of Al^{3+} in the cell nucleus might possibly temporarily prevent the Al^{3+} concentration from increasing in the cytosol. After a certain saturation has been reached in the heterochromatin, the concentration in the cytosol could increase more rapidly and a direct action on the cell membrane and its enzyme systems might be possible. Several of the membrane changes could, theoretically, very likely be caused by an increased Al^{3+} concentrations.

Summing up, Al^{3+} is possibly accumulated in muscle cells in muscular dystrophies and might then be involved in the pathogenesis.

General Discussion

Most of the diseases discussed have not previously been connected with Al^{3+}. They were included here because it was felt that they corresponded reasonably well with the picture of hypothetical Al^{3+}-related diseases, as deduced from the biological effects and metabolism of the ion. For each of them it was remarkably easy to find

reasonable, but purely hypothetical, pathogenic mechanisms based on known Al^{3+} effects. It is also interesting that several of the discussed diseases show a network of epidemiological connections suggesting the existence of common etiological factors (see Fig. 5). Most of the diseases are related to aging, in the sense that they occur in old people and that the pathological processes resemble the normal aging process. The epidemiological connections might, therefore, imply that the diseases are influenced by some of the primary, not well understood, aging mechanisms. The possibility that Al^{3+} accumulation is such a mechanism is the leading theme of the present study.

How Could Al^{3+} Have So Many Different Effects? This study has been made with the full understanding that, from a medical point of view, it must seem absurd to associate a single agent, and even one recently considered completely harmless, with so many disease conditions. Other toxic substances have a few specific effects and are supposed to be bound to defined target structures or receptors. In their case, the affinity and specificity usually depends on extended complementary molecular structures of the toxic substance and its receptor. In this comparison, the Al^{3+} ion is extremely small and devoid of structure. Instead, its affinity depends on a very high charge density causing it to bind, in a biological environment, to almost any oxygen or nitrogen atom. Its charge density is actually nearly maximal; had it been higher, as for its neighbors in the periodic table, boron and silicon, its tendency to bind oxygen would have been so strong that it would cease to be a cation and, instead, would become a complex anion.

The ability to be bound nonspecifically to so many different structures involves, for Al^{3+}, a possibility to affect or interfere with almost every reaction in an organism but also, for the organism, a protection against these toxic effects, as the enormous number of strongly binding sites makes it very unlikely that a given site will actually bind Al^{3+}. For general thermodynamic reasons, however, the ions should tend to move continuously to more stable binding sites and finally to quite irreversible ones, where the concentration might eventually be high. This transfer should be increasingly slow, possibly too slow for the final equilibrium ever to be reached. The main problems are then to know exactly which structures actually accumulate Al^{3+} and at which concentration level this happens. Other questions are in what sequence the possible effects appear and in which time span (effects appearing only when a hundred others have been able to kill the cells are of course less interesting). However, in this field knowledge is almost completely lacking.

The structure best known to accumulate Al^{3+} is the nuclear chromatin and especially the heterochromatin. This accumulation is extremely interesting for several reasons, foremost perhaps, the possibility that it might theoretically cause an increasing heterochromatization. According to an often-held opinion, heterochromatization might, namely, be an essential process in cellular aging (*540–545,939*) but no reliable explanation for it has been proposed. Hence, the possibility seems to be at hand, that accumulation of Al^{3+} in chromatin actually could be one of the fundamental aging mechanisms, especially in long-lived cells.

Why May the Possible Effects Have Escaped Attention? Obviously, any possible disease-causing effects of Al^{3+} have not been much noticed. Some plausible explanations for this fact have already been discussed and are only briefly summarized here.

CONCEPTUAL REASONS: Al^{3+} has generally been considered quite harmless when administered orally, since no effects have apparently occurred even after very large intake. Consequently, very few detailed metabolic or toxicologic investigations have been performed.

Many early investigations have assumed that since Al^{3+} is a normal constituent of the body, it should also have some specific function. Therefore, more efforts were spent on finding this than studying possible toxic effects.

The very sluggish metabolism has not sufficiently been taken into consideration. Effects in humans may appear only after several decades of exposure. In animal experiments a long exposure time cannot be substituted by a high daily dose. Experiments with cell cultures or microorganisms also tend to underestimate the effects as these may be far too slow in relation to the experimental design. Harmful effects in an organ have been thought possible only with a large relative increase in the concentration. The possibility that Al^{3+} might accumulate mainly on a cellular or subcellular level has not been discussed.

TECHNICAL REASONS: Al lacks radionuclides suitable for biological investigations. For other elements access to such nuclides has been a prerequisite for assessing their uptake, distribution, elimination, etc.

Phosphorus interferes with the determination of Al by neutron activation analysis, as ^{31}P and ^{27}Al form the same radionuclide and phosphorus is present in far larger amounts in biological samples.

Histochemical methods have been too insensitive to demonstrate normal amounts of Al^{3+} in tissues, and other ions, e.g., Ca^{2+}, have often interfered. Probably, histochemical methods have underestimated or completely failed to detect crystalline Al^{3+} compounds, e.g., Al^{3+} phosphate.

Usually, microprobe techniques have also been too insensitive to assess normal tissue levels of Al^{3+}, and, in addition, they have often detected only crystalline deposits.

Various chemical methods have certainly been sensitive enough to assay normal tissue levels, but they have provided no information concerning the location of Al^{3+} in the tissues. The methods have often been laborious, and Al^{3+} in the laboratory environment has easily interfered.

EPIDEMIOLOGICAL REASONS: Several of the diseases discussed have been very difficult to study epidemiologically, since the early symptoms—by patients and their relatives—are often mistaken for normal aging and do not result in physician consultations. Due to the insidious onset it has been difficult in population-based investigations to find detection methods for the diseases or to define limit values between the healthy and the

diseased. For maturity-onset diabetes, such attempts have scarcely succeeded, despite many attempts. For other diseases, e.g., SDAT, the result has been far worse.

As an environmental agent, Al^{3+} is also almost impossible to study by epidemiological means. All individuals are abundantly exposed. Intestinal uptake dominates, but absorption might be influenced by special conditions in the intestinal lumen or mucosa more than by the actual intake of Al^{3+}. Observations of the effects of absorbed Al^{3+} may be expected only after years or decades of exposure or may even appear a long time after the exposure. This has made prospective or retrospective investigations impossible or at least very troublesome.

If, indeed, Al^{3+} might have important effects of the types proposed here and in the previous review, it is not unreasonable, for all these reasons, that these connections could have been overlooked.

Final Comment. To avoid any misunderstanding, it must be emphasized that the hypotheses and proposals in this study should not be conceived as indications of new environmental hazards but rather as new, possible explanations of old phenomena.

Appendix: Notes

1. Al^{3+} usually coordinates six atoms of oxygen, when no steric hindrance in the ligand molecules exists, but there is insufficient room for more than six oxygen atoms around the small ion. A ligand exchange therefore requires the formation of intermediates with lower coordination numbers and this entails that one of the former ligands must first leave the complex. The similar but somewhat larger ions Ga^{3+} and Sc^{3+} can form intermediates with coordination number 7, thus enabling ligands of high affinity to squeeze in to the ions. $Ga(H_2O)_6^{3+}$ is reported to have a 10^3–10^4 (*2,940*) and $Sc(H_2O)_6^{3+}$ a 10^8 (*940*) times higher ligand exchange rate than $Al(H_2O)_6^{3+}$.

2. One such way, particularly used by very ancient plant groups (e.g., ferns, horsetails, and club-mosses) (*941–943*), entails Al^{3+} being strongly chelated in the cell vacuole after resorption, whereby the metabolically active parts of the cell are protected. Plants that utilize this method are normally referred to as "aluminum accumulators" (*944*). Another way is to prevent the root uptake, and an important mechanism can be that Al^{3+} is bound to the layer of gelatinous material that is secreted by the roots (mucilage) (*945*). Mucilage consists mainly of polyuronic acid and other polysaccharides that readily bind Al^{3+}. The mucilage layer varies in thickness from a few μm to almost 1 mm and has, in experiments with onions, been shown to accumulate $^{46}Sc^{3+}$ that was used instead of the nonexisting Al-radionuclide. Removal of the mucilage layer markedly increases the root sensitivity to Al^{3+} (*945*).

3. ^{111}In ($T_{½}$ 2.8 days) has mostly been used, and to a lesser extent ^{113m}In ($T_{½}$ 1.7 hr) and ^{114m}In ($T_{½}$ 50 days). Among its many uses in nuclear medicine, various In^{3+} salts and complexes are used to localize tumors. Bone marrow imaging, placenta localization, and cisternography are done with In-transferrin. In^{3+} is used even more extensively for *in vitro* labeling of various cells (e.g., erythrocytes, granulocytes, lymphocytes, and platelets) for studying cell kinetics and cell survival, localization of abscesses and thromboses, etc. In^{3+} is very suitable for labeling cells. The ion is easily taken up as a complex, for example, with 8-hydroxyquinolin or acetyl acetone, and is then bound firmly intracellularly with hardly any radionuclide leakage occurring.

4. Especially in technical products, hexavalent chromium (chromate) occurs which is chemically similar to sulfate. Chromate is readily taken up by organisms but is believed to be immediately reduced in the cells to Cr^{3+} (*412,417,424*). With oral administration, Cr(VI) is seemingly reduced while in the stomach (*375*), but following parenteral administration it is believed to exist extracellularly for a short period before it is taken up by various cells, including the erythrocytes. Cr(VI) is not otherwise discussed in this study.

5. Example. 12 diabetics received GTF, 6 later showed slightly improved glucose tolerance. These were considered to belong to a particular group of "responders" that should, therefore, be treated separately. By rejecting the others, it was shown that administration of yeast extract, with statistical significance, had improved the glucose tolerance of those that responded (*396*).

6. The remaining ions discussed in this review were not investigated. However, the concentration of titanium, the lower analog of Zr, was examined. Sensitivity of the method used was, however, only sufficient for seven organs, but these were the same organs that had the highest Al^{3+} and Cr^{3+} concentrations, and a very high correlation existed between the concentrations of Al^{3+} and Ti^{4+} (r = 1.0). If the values for the lung were excluded, as these were extremely high and therefore far too much biased the correlation, then the correlation coefficient was 0.95. The Ti^{4+} concentration was, on a molar basis, about 6% of the Al^{3+} concentration, both in the lung and in the other organs. This is roughly the same figure as with the average Ti/Al molar ratio in the earth's crust, 4.4%.

7. In a recent review of the literature in this field (*391*), Cr^{3+} is reported to be reabsorbed to 60 to 95%; however, the latter value seems improbably high. If the normal Cr/Al ratio is 1.5% for urine and 2.5% for plasma (see discussion under Cr^{3+}) and the renal clearance for Al^{3+} is 5% of the glomerular filtration rate, then the renal clearance for Cr^{3+} will be 3% of that rate. If 5% of Cr^{3+} in the plasma is ultrafiltrable, only 40% of the Cr^{3+} in the ultrafiltrate can be reabsorbed to result in the given clearance value. If the Cr/Al ratio in urine is somewhat greater, which it apparently is, the space for reabsorption will be even less. Even if all these values are rather uncertain, they appear to imply that a 95% reabsorption is impossible.

8. Excretion of Sc^{3+} in the gastrointestinal tract was, however, no greater totally than for the other ions, instead the reported urinary excretion was remarkably low. Technical complications might explain the differences. Mice excreted over 40% of IV administered trace doses of Sc^{3+}-NTA via the kidneys during the first 24 hr (*284*).

9. The high value can partly be accounted for by the concentration being reported as μg/g ash. Apparently, pure connective tissue results in less ash than more cell-rich tissues.

10. Shown for Ga^{3+} in humans (*214,237*) and rats (*238*), for Sc^{3+} in mice (*282*), for Y^{3+} in rabbits (*292,295*), for Be^{2+} in cows (*312*), for Zr^{4+} in rabbits (*292*), and for Cr^{3+} in rats (*402,403*).

11. Shown for Ga^{3+} in rats (*238*), Sc^{3+} in mice (*282*), Y^{3+} in rabbits (*292,295*), Be^{2+} in cows (*312*), Zr^{4+} in rabbits (*292*), and Cr^{3+} in rats (*402,403*).

12. The daily uptake in the brain could even be lower, as both the value for total absorption of Al^{3+} and the percentage uptake in the brain probably are upper limit estimations. The time required to accumulate 1 mg could, then, be longer.

13. If an active transport mechanism exists, Al^{3+} must pass the endothelial cells and should, probably, be bound there. This could possibly be one of the reasons why a relatively high concentration of Al^{3+} is found in blood vessels (*129,475–477*). The concentration of Al^{3+} in cerebrospinal fluid is reported to be 210 μg/L (*946*), 5–20 μg/L (*110*), 4–15 μ/L (*461*), 35 ± 11 μg/L (*947*), 120 μg/L (*948*) and 2–7 mg/L (*949*). All of these estimations may be too high.

14. If chromatin has the highest affinity for Al^{3+} of all structures in the cells and if uptake of Al^{3+} in the cells and transfer to the cell nucleus cannot completely be avoided by other mechanisms, then it might, instead, be an advantage for the cells to have a special fraction of DNA, that could permanently be held as condensed heterochromatin and could thereby trap any Al^{3+} reaching the nucleus. This could possibly be one of the functions of heterochromatin.

15. In sharp contrast to this generally accepted view, one author has, in several articles (*950,951*) during a 25-year period, argued that Al^{3+} could well be an important factor in the aging process and has even predicted that when cancer and arteriosclerosis can be controlled in the future, accumulation of Al^{3+} could then become the foremost cause of human disease (*952*). These hypotheses have been based on the well-known ability of Al^{3+} to crosslink various macromolecules, notably collagen, and on this author's belief that such crosslinkage of macromolecules is the essential cause of aging. No closer discussion of Al^{3+} metabolism and biochemistry, in other respects, seems to have been published, nor is there any mention of exactly which diseases could primarily be caused by Al^{3+}.

16. The purpose of this section is not primarily to present an historical account of how the role of Al^{3+} in DES was elucidated—for such accounts, reviews on the subject are referred to (*46,89,553,593,953,954*). Instead, the purpose is to give a concise account of the various types of investigations considered jointly to have shown that DES is induced by Al^{3+} poisoning.

17. Several reports of higher (200–700 μg/L) serum concentrations exist, particularly in DES (*68,73,89,96,955*). However, most of these investigations also report higher normal serum values than those reported by the group referred to or being most plausible, as reported in the earlier review of the literature.

18. Purely speculatively, Al^{3+} might interact with silicate. Si is believed to be an essential trace element necessary for normal bone formation. Silicate normally accumulates in the actual mineralization zone without being deposited in the mineralized bone (*956*). It has even been suggested, but (as far as it is known) not substantiated, that silicate contributes to the nucleation. In rats, the normal concentration in the mineralization zone has almost reached 5 mg/g (*956*), which compares extremely well with the Al^{3+} concentrations reported in DOM, 5 mg/g (*613*). (Al and Si have nearly the same atomic weights.) Al^{3+} and silicate form very stable compounds, and there is apparently a possibility that in DOM they accumulate in the narrow mineralization zone in quantities that are almost equimolar and which should mean that their solubility product is greatly exceeded. Hence, such a hypothetical interaction with silicate could possibly explain both the accumulation of Al^{3+} in the zones and its effect on the mineralization process.

19. As a complement to this disease description, see the various review articles and monographs (*551,616–618,628,957,958*).

20. Speculatively, Al^{3+} could be thought in some way to cause tubulin to polymerize as paired helical filaments and not to form microtubules. As an interesting antiparallel, G-actin, which is believed to be distantly related to tubulin, normally polymerizes as paired helical filaments to F-actin, but can instead, under the influence of the Al^{3+} analogs Y^{3+} and Sc^{3+}, form larger tubular structures reminiscent of microtubules (*290*).

21. Zr(VI) is more acidic than Al(III) and at pH 7.4 exists, to about 95%, as pentahydroxozirconate, $Zr(OH)_5^-$, which is not prone to form complexes (*3*). At pH 8–9, which probably may occur in the outer skin layer after, for example, using soaps, Zr exists almost totally in this form. For Al(III) to exist, to the same extent, as $Al(OH)_4^-$, a pH is required in the range of 10–11. Such a high pH possibly causes tissue damage and may, therefore, inhibit immunologic reactions and explain why wet cement, for example, does not give rise to Al^{3+} hypersensitivity or contact dermatitis. For Be^{2+}, it has been shown that BeF_2 sensitizes and provokes hypersensitivity symptoms much easier than other Be salts (chloride, sulfate, and nitrate) (*344,857,959*). BeF_2 is hygroscopic and readily soluble in water, but the ionic complex is, at the same time, so stable that BeF_2 is a very weak electrolyte in water solutions (*300*). Therefore, BeF_2 does not form complexes with anions and shows no tendency to add neutral ligands to itself (*300*). BeF_2 can therefore easily penetrate the skin, but Be^{2+} ions are released slowly, giving rise to allergens. BeF_2 is one of the most common Be^{2+} compounds used technically, as it holds a key position in the production of metallic Be. The corresponding AlF_3 is also very stable and technically commonly used, but, compared to BeF_2, it is poorly soluble in water.

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