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ALUMINUM IN THE ORGANS AND DIET OF AGEING C57BL/6J MICE

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SUMMARY

Total aluminum concentrations increased with ageing in the liver and kidney of male C57BL/6J mice, remained unchanged in brain and heart, and decreased with ageing in femur and lung for mice ranging in age from 56 to 1186 days. Ligating one kidney did not significantly increase aluminum concentrations in the various organs. Feeding 1×10^{-2} M aluminum chloride (270 ppm Al) in the drinking water beginning at 604 days of age decreased the average life span by 6.7%. We conclude that very little aluminum accumulation occurs with ageing in the organs tested in this study, in spite of a high dietary intake. Other organs might show a change. Only one aluminum concentration was used in this study which accelerated the rate of ageing as indicated by a change in the survival curve. The effect of higher or lower aluminum concentrations remains to be seen.

Key words: Longevity; Mice; Aluminum

INTRODUCTION

We have previously reported that aluminum accumulates during development and ageing of *Drosophila* and that excess dietary aluminum increases *Drosophila*'s rate of ageing [1]. In view of the widespread use of aluminum salts in medicine and food processing it seemed desirable to know if a similar result would be found in a mammalian system. Here we report that aluminum accumulates with ageing in some organs of mice while decreasing in others and that excess dietary aluminum reduces the survival times for mice just as it does for *Drosophila*.

MATERIALS AND METHODS

Biological sample and diet

Male C57BL/6J mice obtained from Jackson Labs., Bar Harbor, ME were used for all experiments. Mice were purchased at 1 month of age and introduced into our

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colony. Purina laboratory chow (No. 5001) and tap water were given *ad libitum* to the ageing colony. According to the label the Purina laboratory chow contained 23.4% protein and 4.5% fat. Animals were kept at 22°C and lights were on 12 h and off 12 h.

Survival study

Mice were removed from our ageing colony at 604 days of age and placed 7/cage in plastic cages with stainless steel tops. Corncob bedding and distilled water bottles were changed weekly. The control and experimental groups each consisted of 14 mice. The control group received distilled water and the experimental group 0.01 M $AlCl_3$ (Baker Chemical Co.) in the drinking water. Mice were weighed monthly. All animals were allowed to eat Purina Laboratory chow (No. 5001) without restriction. Cages were monitored daily for deaths.

Aluminum determination

Mice were removed from our ageing colony at various ages, sacrificed and the organs perfused with 0.1 M HEPES buffer in order to remove blood. Organs were dried at 88°C overnight and the dry weight of each organ determined prior to digestion in Ultrex Nitric Acid (J.T. Baker Chemical Co.). Complete digestion usually required 1 week at room temperature. Samples were then diluted with deionized water prior to analysis at 309.3 nm on a Varian 1250 atomic absorption spectrophotometer with carbon-rod atomizer Model 90. Hydrogen gas carrier was used in order to maximize the detection limit.

Kidney ligation

Mice of between 545 and 552 days of age were anesthetized by ether inhalation. One flank was shaved and an incision made to expose one kidney. The renal vein and artery were permanently occluded by ligation with a "oo" silk suture. Immediate blanching of the renal cortex was used as the indicator of effective occlusion.

Diets and components

Dr. E.P. Les provided us with the major diets (911A, 96WA and Wayne) used by the Jackson Laboratory. Stephen P. Cail provided us with Purina Feed which was fed to the National Institute on Aging mouse colonies. Dr. Damon Shelton of the Ralston-Purina Co. generously sent us components used for the manufacture of Purina mouse food.

NIA mice

Mrs. Jane L. Soban sent us the oldest available C57BL/6J male mice from the NIA colony. Only 3 of the 5 mice survived shipment. These mice were reported to be 39 months of age which we converted to 1186 days of age. The mice were maintained by Charles River Labs. Inc. on Purina chow No. 5012-7.

RESULTS

We measured aluminum concentrations in the organs of C57BL/6J male mice ranging in age from 63 to 1186 days of age. The greatest amount of aluminum was found in femur and the least in kidney. Aluminum increased with age in liver and kidney, remained unchanged in brain and heart and decreased with age in femur and lung. In general the data were more scattered than we have observed for other metals in biological tissues. Tipton and Cook [2] have previously noted this for aluminum in human tissues where they found that "in any one tissue the variation in the concentration of aluminum from sample to sample was wide".

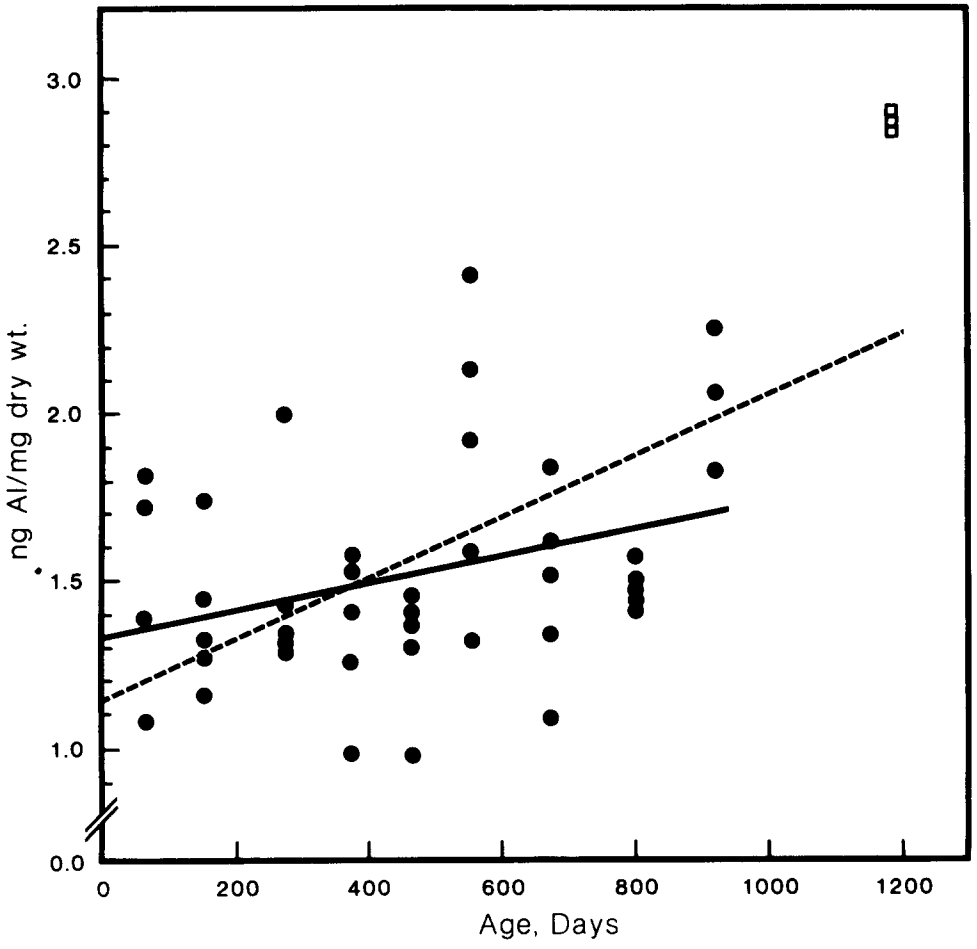

Fig. 1. Aluminum content of mouse liver *vs.* age. Aluminum is expressed as ng/mg of dry weight. Solid circles (●) indicate values for individual mice from our ageing colony (MMRL). Solid line is least squares fit for colony mice. Open squares (□) indicate NIA mice and dashed line least squares fit for NIA plus MMRL mice.

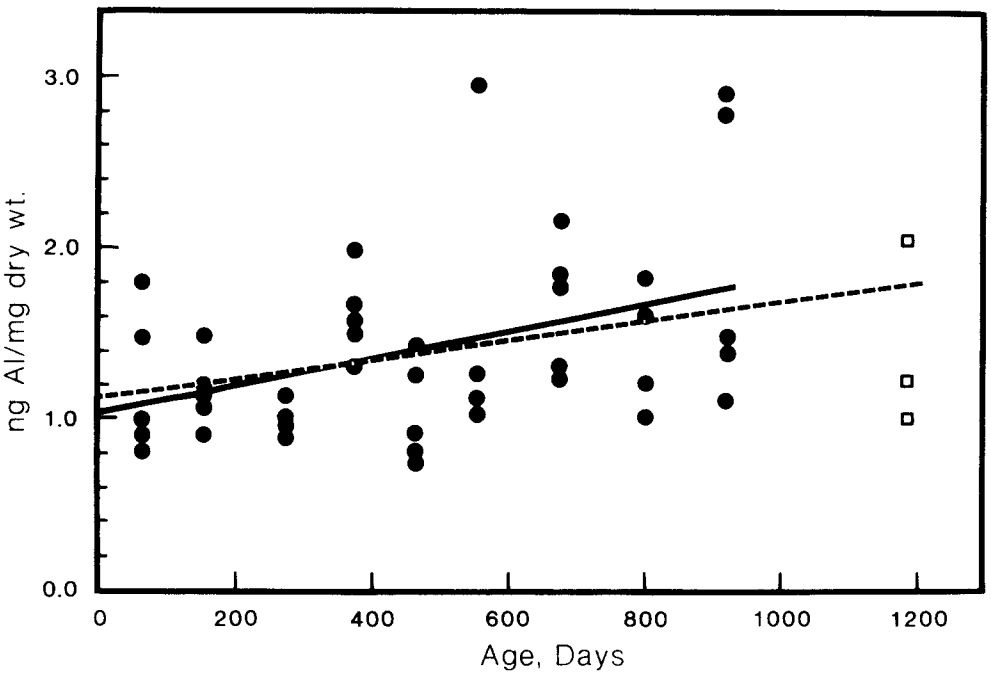

Fig. 2. Aluminum content of mouse kidney *vs.* age. ●, MMRL mice; □, NIA mice.

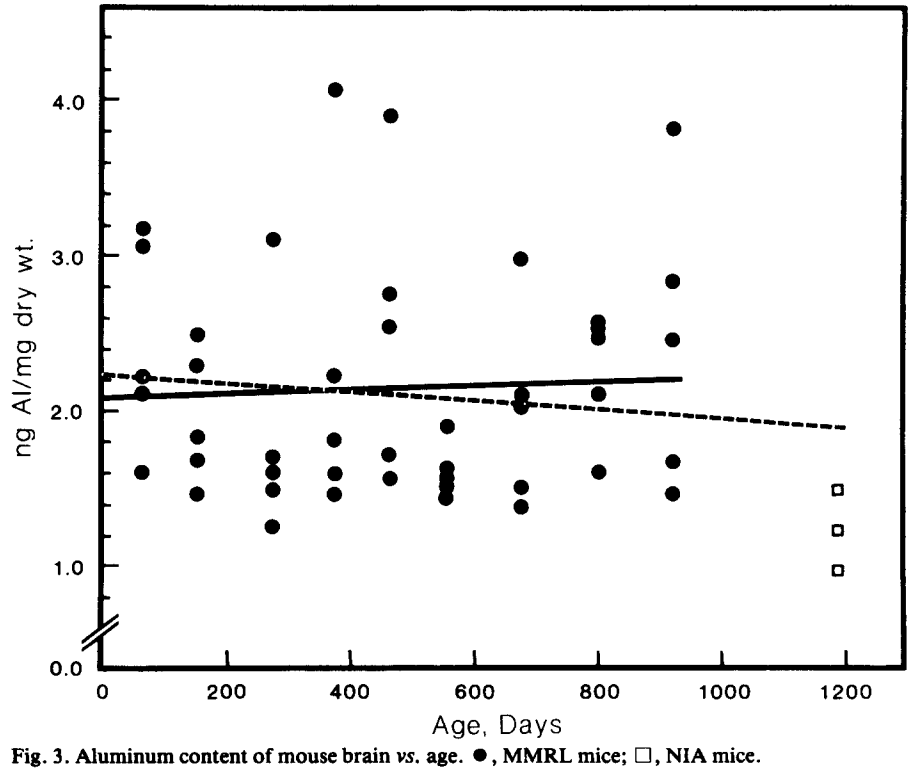

Fig. 3. Aluminum content of mouse brain *vs.* age. ●, MMRL mice; □, NIA mice.

Liver

Liver was the organ showing the greatest increase in aluminum concentration with ageing. Two least squares fit lines are drawn in Fig. 1. The solid line fits the data for the mice from our colony (MMRL) and the dashed line fits the data for MMRL mice plus 3 very old (1186 days) mice from the National Institute on Ageing colony (NIA). The least squares fit line for the MMRL colony was, Al = 0.000403(age) + 1.34 where age was in days and aluminum (Al) was expressed as nanograms per milligram of dry weight. The correlation coefficient was 0.322, number of mice = 42 and $0.01 < P < 0.05$. The least squares fit line for MMRL plus NIA mice was Al = 0.000900(age) + 1.152 (correlation coefficient = 0.606, $n = 45$, $P < 0.001$. The increase with ageing of hepatic aluminum was, therefore, significant for both sets of data.

Kidney

The increase in renal aluminum with ageing was also significant for both sets of data. For the MMRL mice from 63 to 919 days of age the least squares fit line was, Al = 0.000805(age) + 1.03 (correlation coefficient = 0.418, $n = 42$, $0.001 < P < 0.01$). For MMRL plus NIA mice from 63 to 1186 days of age the least squares fit line was, Al = 0.000569(age) + 1.11 with a correlation coefficient of 0.347, $n = 45$ and $0.01 < P < 0.05$.

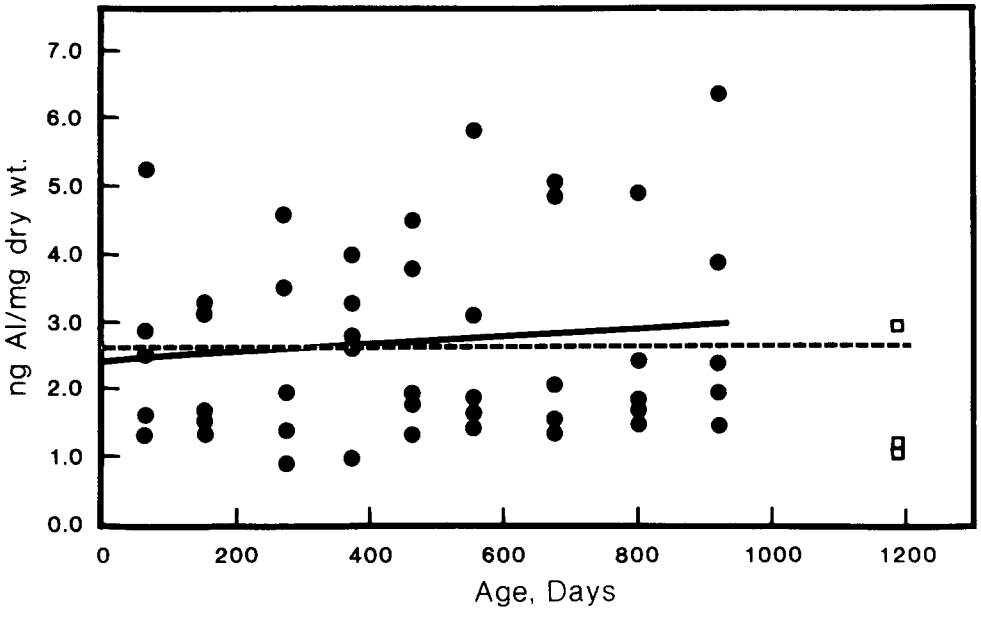

Fig. 4. Aluminum content of mouse heart *vs.* age.

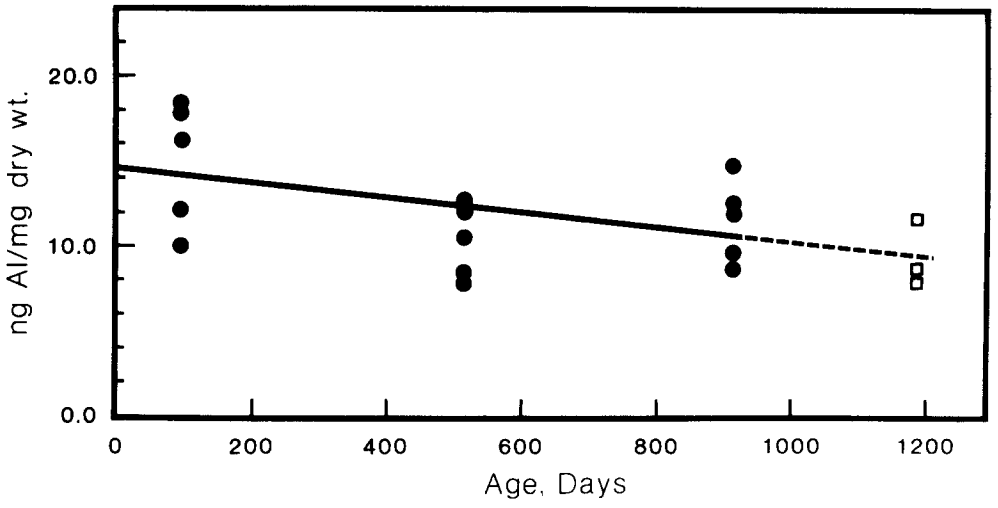

Fig. 5. Aluminum content of mouse femur *vs.* age.

Brain

Surprisingly, brain showed no change with ageing for either set of data. The least squares fit for MMRL mice from 63 to 919 days of age was, Al = 0.000138(age) + 2.08 (correlation coefficient = 0.0536, $n = 45$, $P > 0.05$). For MMRL plus NIA mice the least squares fit was, Al = −0.000282(age) + 2.24 with a correlation coefficient of −0.124, $n = 48$, $P > 0.05$.

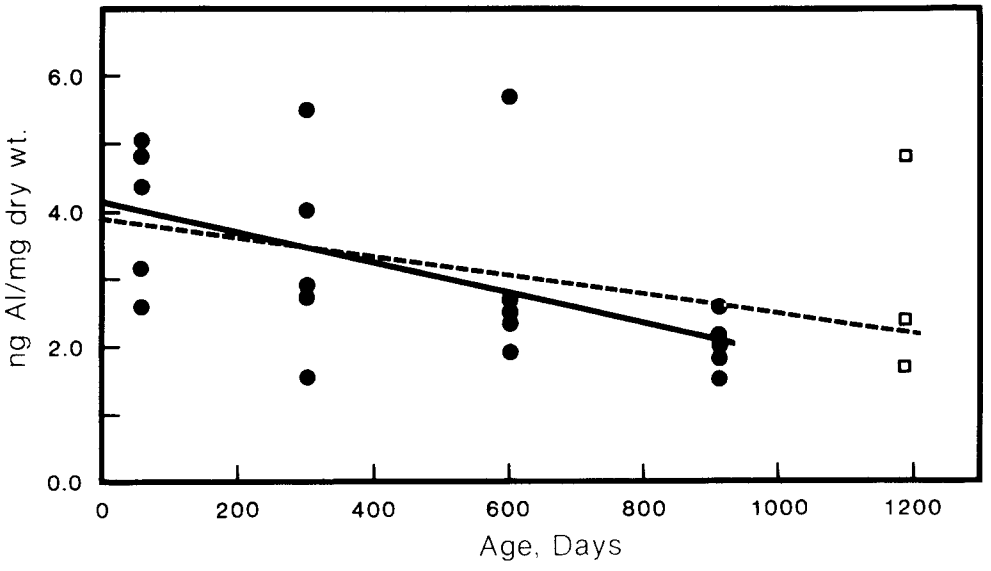

Fig. 6. Aluminum content of mouse lung *vs.* age.

Heart

The amount of aluminum in heart was also unchanged with ageing. For the MMRL mice, Al = 0.000629(age) + 2.40 (correlation coefficient = 0.122, $n = 45$, $P > 0.05$) and for the MMRL plus NIA mice, Al = 0.0000373(age) + 2.62 (correlation coefficient = 0.0084, $n = 48$, $P > 0.05$).

Femur

By far the highest concentration of aluminum was found in the femur. The least squares fit for our colony (MMRL) mice was, Al = −0.00416(age) + 14.5 (correlation coefficient = −0.436, $n = 15$, $P > 0.05$) for mice from 93 to 912 days of age. When the 3 NIA mice were included the least squares fit was, Al = −0.00418(age) + 14.5 (correlation coefficient = −0.522, $n = 18$, $0.01 < P < 0.05$), indicating a significant decrease with ageing.

Lung

Another surprising result was the decrease in aluminum content with ageing in lung. The decrease was significant for our colony mice, Al = −0.00221(age) + 4.13 (correlation coefficient = −0.548, $n = 20$, $0.01 < P < 0.05$), where the mice ranged in age from 56 to 910 days. Including the 3 NIA mice gave a least squares fit of, Al = −0.00140(age) + 3.87 with a correlation coefficient = −0.415, $n = 23$, $0.01 < P < 0.05$.

Food aluminum content

The mouse food used in this study was Purina chow No. 5001. The aluminum content of this food was 289.1 μg Al/g dry wt. The diet used for the NIA mice at Charles River was also from Purina (No. 5012-7) and contained 217.7 μg Al/g dry

TABLE I

ALUMINUM CONTENT OF COMMERCIALLY AVAILABLE MOUSE FOOD

Food sample	*μg Al/g dry wt*
Purina rodent laboratory chow No. 5001	289.1
ICN Nutritional Biochemical custom low iron control diet	2.1
911R Old Guilford mouse diet (from Emory Morse Co.)	25.2
911A Old Guilford mouse diet (from Jackson Lab.)	24.4
96WA Old Guilford mouse diet (from Jackson Lab.)	32.0
Wayne sterilizable rodent blox mouse diet (from Jackson Lab.)	179.7
96W Old Guilford mouse diet (from Emory Morse Co.)	29.4
Purina No. 5002 certified lab checkers (from Purina)	63.0
Charles River rat and mouse food No. 5012-7 (from Purina)	217.7
Charles River rat and mouse food No. 5012-7 (from Charles River)	203.8

TABLE II

ALUMINUM CONTENT OF VARIOUS COMPONENTS OF PURINA MOUSE FOOD

Purina rodent laboratory chow No. 5001 components	*μg Al/g dry wt*
2910 No. 1 Fine alfalfa	85.9
2940 Dehydrated alfalfa	91.7
2780 Ground wheat	10.5
2150 Ground fine corn	4.4
3050 Ground beet pulp	470.5
3800 Brewers yeast	15.1
3910 Wheat middlings	20.5
4930 Fish meal	308.1
5000 Meat meal	143.1
5610 Calcium carbonate	225.3
8695 Trace mineral L	59 826.6

wt. We also tested other commercially available rodent foods for their aluminum content (Table I). The values ranged from 2.1 to 289.1 μg Al/g dry wt.

In view of the relatively high aluminum content of Purina mouse food we analyzed the various components of chow No. 5001 for aluminum and found the major sources to be ground beet pulp and the trace mineral mixture (Table II).

Kidney ligation

Since renal function is known to decline with ageing [3] and the major pathway for aluminum excretion is through this organ, we tested the possibility that reduced renal capability might lead to an increase in aluminum content of other organs. One kidney was ligated and the mice allowed to consume Purina chow containing 289.1

TABLE III

ALUMINUM CONTENT OF ORGANS FROM MICE WITH ONE KIDNEY LIGATED FOR 12—13 DAYS

Mice were 545—552 days of age.[a]

Organ	*Al (ng/mg dry wt)*		*P*
	Ligated	*Sham operated*	
Brain	2.26 ± 0.54	2.55 ± 0.60	>0.05
Heart	4.50 ± 1.05	3.85 ± 1.73	>0.05
Kidney	2.50 ± 1.31	1.35 ± 0.52	>0.05
Liver	2.04 ± 0.29	1.75 ± 0.31	>0.05
Lung	3.43 ± 1.82	2.34 ± 0.29	>0.05

[a]There were 4 mice in the sham operated group and 5 in the ligated group. Aluminum values are averages ± standard deviation.

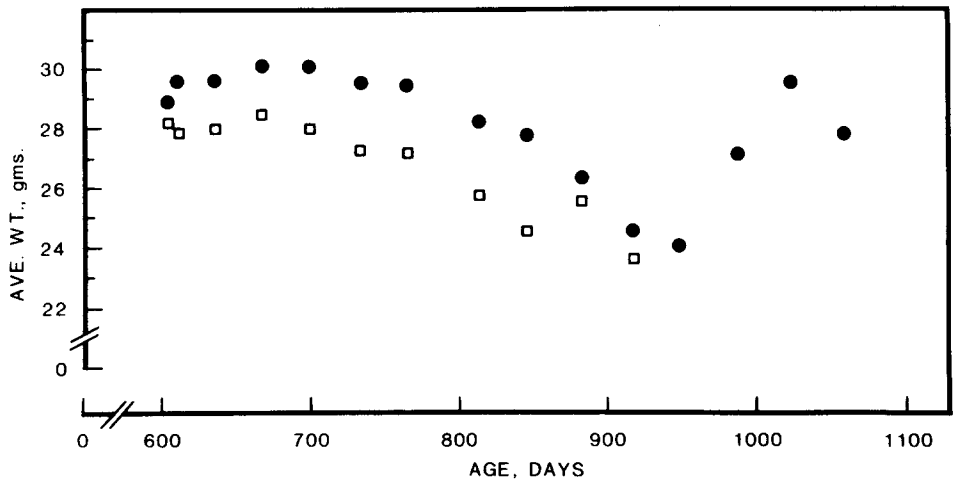

Fig. 7. Average weight *vs.* age for mice drinking distilled water (●) and water containing 270 ppm $AlCl_3$ (□) beginning at 604 days of age.

μg Al/g dry wt for 12—13 days. We used this period of time in order to avoid the increased kidney function which occurs as a result of hypertrophy of the remaining kidney. All of the organs except brain showed increases in aluminum concentration but none of the differences were significant ($P > 0.05$). It, therefore, seems unlikely that reduced kidney function is a major contribution to elevated tissue concentrations of aluminum under normal conditions.

Survival study

Our previous studies with *Drosophila* demonstrated that increased dietary aluminum reduced survival times for *Drosophila* [1]. We did a small pilot experiment where 1×10^{-2} M aluminum chloride (270 ppm Al) was given in the drinking water to C57BL/6J male mice beginning at 604 days of age. The mice were also given without restriction Purina chow containing 289 ppm aluminum. Since the mice consumed about 5 g of food/day and drank approximately 5 ml of water/day, adding 1×10^{-2} M $AlCl_3$ to the drinking water resulted in a doubling of the normal aluminum intake.

Figure 7 shows that the group drinking the aluminum chloride water maintained a lower body weight for most of their remaining life span. The aluminum chloride group also died at younger ages (Fig. 8) with a mean age of 923 days for the control group and 859 days for the aluminum group. When all of the dates of death were analyzed by Student's *t*-test, the difference between the means was not significant ($P > 0.05$, $t = 1.967$). The lack of significance was due to the large standard deviations (86.2 for the control and 79.4 for the $AlCl_3$ group) resulting from one

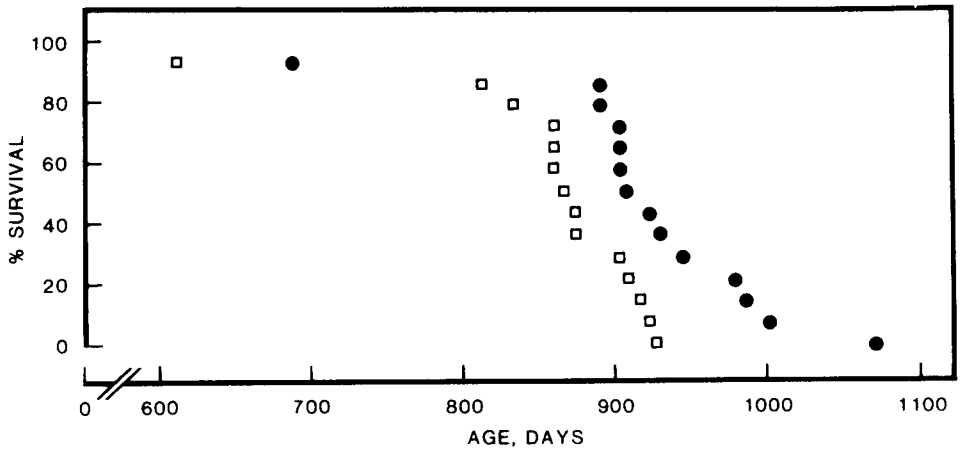

Fig. 8. Survival curves for mice given distilled water (●) and water containing 270 ppm $AlCl_3$ (□) beginning at 604 days of age.

early death in the aluminum group at 610 days and one in the control group at 686 days. When these 2 early deaths were removed, the means and standard deviations were 941 ± 54.8 and 878 ± 35.4 for the control and aluminum groups, respectively, and the differences became significant ($0.001 < P < 0.01$). We believe that removing the 2 early deaths is justified but it is also clear that a life-time survival study involving a larger number of mice would be desirable.

DISCUSSION

It is remarkable that the aluminum concentrations in biological tissues are so low when we consider that aluminum is the third most abundant element in the earth's crust. We found the greatest amount of aluminum in bone with average values of less than 15 ppm. Tipton and Cook [2] reported much higher values for human skin and lung. Their median value for lung on a dry weight basis was 94 ppm while our highest value for lung was less than 6 ppm. Underhill and Peterman [4] also reported high values for aluminum in the lungs of old dogs while finding little or none in young dogs. They proposed that the source of the aluminum was the dust in the atmosphere. Apparently atmospheric aluminum was low in our mouse colony. We also found that lung aluminum concentrations actually decreased with ageing. A more recent report of lung aluminum versus age in humans gives an average of 56 ppm and an increase with ageing [5]. This suggests that the human exposure to atmospheric aluminum may be declining.

The brain was another organ where we had expected to find an increase in aluminum concentrations with ageing. There was no change with ageing in

agreement with the report of Delaney [6] of no change in the aluminum content of human spinal fluid with ageing. Two different laboratories, however, have demonstrated an increase with ageing in human brain concentrations of aluminum with the major part of the increase occurring after 70 years of age [7,8]. We had expected that the very old mice (1186 days) from the NIA colony would prove to have elevated aluminum levels in the brain but clearly they did not (Fig. 3). Accumulation of aluminum in the brain with ageing does not, therefore, appear to be a general phenomenon.

Liver and kidney were the only organs to give an increase in aluminum concentrations with ageing. The increases, however, were of a small magnitude. Aluminum in the heart did not change with ageing and aluminum in femur and lung actually declined with increasing age. Decreasing kidney function by ligating one kidney also failed to significantly change the aluminum content of the mouse organs. It is known that aluminum ions are required for the regulatory component of adenylate cyclase by fluoride ions [9] and that aluminum may have a nutritional role based on the hypocholesterolemic effects of aluminum-polysaccharide complexes [10]. It, thus, seemed possible to us that while the aluminum content of Purina chow was relatively high, it may not have been adequate on a long term basis. Doubling the aluminum intake by adding 1×10^{-2} M aluminum chloride to the drinking water of 604-day-old mice did not, however, improve their survival. One obvious difference between the control and aluminum group was a weight loss in the mice receiving the aluminum chloride (Fig. 7). The weight loss was maintained for the remainder of their life span and was significant ($0.01 < P < 0.05$). Ellis *et al.* [11] have reported a similar decrease in body weight for rats injected with aluminum chloride over a period of 50 days. The mechanism is unclear.

Excess dietary aluminum does appear to reduce the life span of mice but the reduction was not great (6.7%) and it was significant only when 2 early deaths were removed from the analysis. It should be stressed that we did not begin the aluminum feeding until the mice were 604 days of age. A longer feeding time may have increased the reduction in life span. Feeding 1×10^{-2} M $AlCl_3$ to *Drosophila* for their entire adult life span reduced the median survival time by 15.1% [1]. The degree of susceptibility to long-term exposure to aluminum ions, thus, appears to be similar for both *Drosophila* and mice. Whether or not the amount of aluminum in commercially available chows contributes to the rate of ageing remains to be established. Of particular concern is the possibility that when certain chows are used in dietary restriction experiments the intake of aluminum might be altered (Purina chow is commonly used in these experiments). We are currently feeding commercially available chows with different aluminum contents in order to evaluate their influence on life span.

There is no doubt that high intakes of aluminum salts can produce osteomalacia [12—16] and dementia [17,18] in humans. The mechanism for these changes and the reduction of survival reported in this study remains unknown. One possibility is that

aluminum ions prevent the absorption of other ions and nutrients in the gastrointestinal tract. The average daily intake of aluminum for humans is less than 20 mg with only 5% being absorbed [19]. This is considerably below the amount present in most rodent chows. It, thus, seems unlikely that the low dietary intake for humans would have an unfavorable effect on human life span. The high concentrations of aluminum in human lung, however, may represent an alternate and more important point of entry into the body. This may explain why the concentration of aluminum increases with ageing in the human brain but not in that of the mouse.

ACKNOWLEDGEMENT

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