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# Behavioral Effects of Aluminum Ingestion On Animal and Human Subjects[1,2]

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BOWDLER, N C, D S BEASLEY, E C FRITZE, A M GOULETTE, J D HATTON, J HESSION, D L OSTMAN, D J RUGG AND C J SCHMITTDIEL *Behavioral effects of aluminum ingestion on animal and human subjects* PHARMAC BIOCHEM BEHAV 10(4) 505–512, 1979 —Abnormally high brain aluminum concentrations have been detected in hemodialysis patients who died of an unexplained encephalopathy As a result, this study was undertaken to examine whether the ingestion of aluminum produces behavioral aberrations in non-dialysed human subjects and rats with ostensibly normal renal function Rats were fed $AlCl_3$ by intubation in varying doses, and tests measuring learning ability, visual temporal acuity, motor coordination and activity were administered It was found that orally ingested aluminum is absorbed by rats and deposited in the brain High brain aluminum levels are associated with rapid general activity, decreased ability to maintain roto-rod activity, and increased sensitivity to flicker Behavioral tests were also given to elderly human subjects and performance correlated with serum aluminum level High serum levels of aluminum in elderly humans are associated with impaired visuo-motor coordination, poor long-term memory, and increased sensitivity to flicker

Aluminum Behavior Toxicity Antacids

PRIOR research has suggested that aluminum ingestion may be causally related to various neurological and behavioral abnormalities. Several studies of hemodialysis patients receiving aluminum-containing phosphate-binding gels have reported patients to develop a progressive form of dementia which results in death [1, 8, 12]. The dementia is characterized by such clinical symptoms as paranoia, confusion, impaired ability to perform numerical tasks, and delirium [12]. In one investigation of patient deaths preceded by encephalopathy, unusually high levels of aluminum were detected in the patients' muscle, bone and brain tissue [1] An analysis of cerebral grey matter revealed an aluminum level of 25 parts per million (ppm) in the dialysis patients with the encephalopathy, as opposed to 6.5 ppm in dialysis patients who had died of other disorders. A control group of non-dialyzed subjects was found to have a level of 2.2 ppm. From these results, the authors concluded that the encephalopathy may have been related to aluminum intoxication.

Studies employing animal subjects found that oral and parenteral administration of aluminum salts resulted in aluminum intoxication characterized by periorbital bleeding, lethargy, anorexia and death. For both nephrectomized and non-nephrectomized rats given daily aluminum salt treatments, elevated aluminum levels were reported in plasma, liver, heart, striated muscle, brain and bone [2]. Another study [14], had shown orally ingested aluminum to be deposited in measurable amounts in the tissues of rats, especially when administered as aluminum hydroxide, a major component of several commercially available antacids for human use.

Aluminum ingestion has also been shown to be associated with neurological and behavioral disorders in normal animal subjects. A team of investigators [6,7] found aluminum to induce neurofibrillary degeneration (NFD) when injected into the hippocampus, entorhinal cortex, and neocortex of cats. Another study [10] reported an absence in the cat visual cortex of neurons with spontaneous firing frequencies between 7 and 12 spikes per second 10 days after hippocampal injections of $AlCl_3$ This finding was attributed as an effect of NFD caused by elevated aluminum concentrations in the lateral gyrus

The clinical features observed in the cats of these studies were similar to those seen in patients with Alzheimer's disease, which is characterized by dementia It was noted that some brain regions in the victims of Alzheimer's disease contained raised aluminum concentrations, particularly the regions of the cerebral cortex likely to exhibit NFD [1,9].

That patients with Alzheimer's disease also showed an

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aluminum-related encephalopathy suggests that aluminum uptake may result from sources other than dialysis therapy One study [1] reported that an aluminum flake-powder factory worker developed an encephalopathy and was found to have a brain aluminum level of 20 ppm Since aluminum is found in the environment, human diet, and in many commercial antacid preparations, it is possible that individuals might ingest enough aluminum during the course of their lives to cause behavioral or neurological impairment

The present study is an attempt to investigate the relationship of aluminum intake and behavioral and neurological functioning in both human and animal subjects Therefore, given the evidence that (a) aluminum is toxic to animals when administered in large doses, (b) ingested aluminum is absorbed into the tissues of humans and rats with normal renal function, and (c) behavorial and neurological manifestations are associated with aluminum-induced NFD, the present study addressed the hypothesis that: administered either in high doses, or over considerable periods of time, aluminum salts will produce behavioral manifestations in non-dialyzed subjects, such as decreased learning ability, impaired motor coordination, and visual aberrations

## METHOD

### *Human Study*

*Subjects.* Ninety-three male and female volunteers between the ages of 56 and 90 years, who were participating in various senior citizens organizations in the south-central Michigan area took part in the study They were ambulatory and in good general health; none was from a nursing home population Education levels varied from partial completion of grade school to four years of college

*Instruments and apparatus* Demographic information was secured by means of a questionnaire which included factors such as age, education, occupation, medical history, antacid consumption and other medicines taken regularly Dosages and lengths of usage of all medications were assessed

The Trail Making Test [19], an indicator of organic brain damage, was employed to measure visual-conceptual and visuo-motor tracking The Serial Sevens Test [15] was used as a test of mental tracking.

Several tests were selected from the Wechsler Adult Intelligence Scale (WAIS) [20] including the Digit Symbol Test to assess visuo-motor coordination, the Block Design Test to measure visuo-spatial organization, and the Digit Span Test an indicator of short term memory

Visual-temporal acuity was measured using an apparatus for determining critical flicker frequency [3] The flicker stimulus was a 0 5 cm amber light-emitting diode (LED) (Monsanto MB5352, with an intensity of 0.4 log foot Lamberts) driven by a square-wave generator (all measurements of light intensity were made with a SEI exposure photometer which was calibrated against a Spectra brightness standard) The diode was situated at one end of a 45 7×45 7×40 6 cm box The box contained a partition, 10 cm from the face of the apparatus Holes of diameter 7 9 cm were drilled in the center of the partition and the face of the box The box was painted flat black to minimize reflection and four 10 watt incandescent bulbs (with a combined intensity of 0.2 log foot Lamberts) were placed symmetrically around the viewing hole on the partition inside the box to uniformly illuminate the back of the box The frequency of flicker was varied by means of a dial and push-button system, with flicker rates calibrated to range from 27 to 45 Hz

*Procedure* The subjects were informed as to the nature of the study and informed consent was obtained for the behavioral tests and collection of a blood sample The subjects then completed the questionnaires

Standard procedures were followed for administration of the Trail Making Test, the Serial Sevens Test, and the three tests from the Wechsler Adult Intelligence Scale

Prior to commencing the flicker fusion test, the subjects fixated on the stimulus for three minutes to adapt to the amount of light inside the apparatus They were then shown a stimulus that clearly appeared to be flickering and were asked to turn the dial controlling the frequency of flicker until the light appeared to cease flickering This initial threshold frequency was noted and the random double-staircase method [5] was then used to determine the flicker fusion threshold Staircases were commenced at the threshold level obtained with the adjustment method Subjects made binocular discriminations for 56 trials

A 2 ml sample of blood was then collected by venipuncture. The serum was later analyzed for aluminum concentration by atomic emission spectrometric techniques [16]

### *Rat Study*

*Animals* The animal study was performed in two phases In Study 1, which was terminated after three weeks because of deaths in the rat population, 60 rats were utilized. In Study 2, lasting four weeks, 75 rats were employed for testing and an additional 30 rats were used for various control studies All of the animals were male albino Sprague-Dawley rats and were 60 days old upon receipt. Rats in various treatment groups were randomly assigned to group cages The cages containing animals utilized for testing were placed in a room which was maintained in constant darkness at 25°C There now exists evidence [4] of retinal deterioration in albino rats due to prolonged exposure to fluorescent lights Avoiding this was seen as essential for electroretinogram testing

### *Apparatus*

*Open field measure* The open field apparatus was patterned after that employed by Price and Huck [18] and consisted of a circular plywood base, 1 8 m in diameter, with a sheet metal wall, 46 cm high The base was divided into 24 sectors by five concentric circles of radii 18, 37, 55, 73, and 91 cm, and a series of radial lines The interior was painted white with black circular and radial lines The open field was illuminated by red light, and was housed in a 2 7 by 2 7 m room at 25°C The movements of each rat were followed on a video monitor from an adjoining room and were recorded using stop watches and counters The open field maze was employed as a measure of general activity

*Roto-rod* The roto-rod was patterned after an apparatus used by Overman [17]. The apparatus (length 25 8 cm) was covered with emery cloth to provide traction for the animals The rod was driven by a 60 cycle AC synchronous motor A burlap catch net was placed 90 cm below the roto-rod In Study 1 a drum of diameter 8.4 cm was rotated at 12 revolutions per minute (rpm) and for Study 2 a drum of diameter 5 3 cm was rotated at 26 rpm

*Electroretinogram* The animals were secured in a stereotaxic apparatus equipped with ear pins and bite bar ERG's were recorded with cotton wick electrodes made

from saline-filled glass pipettes containing coiled chlorided silver filaments. An amber LED (Monsanto MB5352) with an average intensity of 1.3 log foot Lamberts served as the flickering stimulus and was driven by a variable speed square-wave generator. The rats were monocularly tested, with the stimulus placed 2 cm from the animal's right eye The rate of flicker ranged between 4 and 40 Hz The responses were monitored on a Tektronix Type 3A9 oscilloscope, with 3 dB cutoffs at 0.1 Hz and 300 Hz.

*Shuttle box* The shuttle box was a 14×97×36 cm wooden box with a clear Plexiglas top. One half of the inside of the box was painted black and was covered to prevent light from entering. The other half of the box was painted white and illuminated from above A door which could be raised by means of a pulley separated the black and white areas of the shuttle box A manually operated 0.5 mamp Grason-Stadtler shock generator was connected to a wire grid that made up the floor of the black portion of the box. The box was located in a darkened room at a temperature of 25°C.

*Procedures*

*Administration of aluminum* Aluminum was administered to rats 7 days a week by intubation, using a solution of $AlCl_3$ $6H_2O$ in tap water. The daily dose for all rats was divided between two feeding sessions in an attempt to increase absorption of aluminum. To facilitate intubation, ether was administered prior to each feeding, until the rat lost its righting reflex All rats were weighed every 72 hr and the dose of $AlCl_3$ $6H_2O$ was based on the most recent weighing

The 60 Group 1 rats were divided into four groups of 15 which received control, low, medium, and high doses of 0, 550, 1100, and 1650 mg $AlCl_3 \cdot 6H_2O$/kg body weight/day respectively, for 21 days. Each of these rats received a volume of 28.6 ml/kg/day body weight. The osmolalities of the solutions for Group 1 were 14, 292, 612, and 939 mOsm/kg with pH's of 7 21, 3.40, 3.04, and 2.81 for the control, low, medium, and high solutions respectively

Doses for 60 of the Group 2 rats (similarly divided into four groups) were 0, 200, 400, and 600 mg $AlCl_3$ $6H_2O$/kg body weight/day for 28 days The control rats were fed a solution with an osmolality of 14 mOsm/kg while rats receiving low, medium, and high doses were fed varying volumes of one solution having an osmolality of 231 mOsm/kg Both solutions had a pH of 7 21 (All pH determinations were made by Heath EU-302A pH-voltmeter, and osmotic concentrations were determined by Wescor 5100 B vapor pressure osomoter ) The rats in the low group received a volume of 11 4 ml/kg body weight/day, whereas the rats in the medium and high groups received volumes of 22 9 and 34.4 ml/kg body weight/day respectively.

In addition, 16 rats were given a solution of a commercial antacid preparation containing $Al(OH)_3$ (Wyeth-Amphojel), which had a pH of 7 05. These rats received 195 mg $Al(OH)_3$/kg body weight/day in a volume of 24 3 ml to yield a dose of elemental aluminum equivalent to that received by the high dose animals in Group 2 This experiment was performed in an attempt to assess whether the uptake of aluminum from antacids approximates that of $AlCl_3$. These rats were housed in a constantly lighted room at 25°C for most of the study Behavioral tests (including the ERG) were administered to these rats after four weeks of Amphojel ingestion. Twenty-four hr before testing these animals were placed in constant darkness

In an attempt to ascertain whether any of the deaths in Group 1 were due to hyperosmolality or acidity of the feeding solutions, a study was initiated in which NaCl of varying concentrations and acidity were administered to rats Seven rats were fed a solution of NaCl of concentration 891 mOsm/kg and pH 7.21, and seven rats were fed NaCl of 899 mOsm/kg and pH 2 81.

Finally, fifteen rats were added to the high $AlCl_3$ dose group of Group 2 in anticipation of an elevated mortality rate among these rats

*Blood and brain samples* Baseline blood samples were obtained from Group 1 rats by heart puncture At the end of the study both Group 1 and Group 2 rats were anesthetized with ether and decapitated. Blood samples were obtained at this time The blood was centrifuged and the serum frozen for later analysis Upon sacrifice, the brains of all rats were removed and frozen for subsequent analysis of aluminum content by Atomic Emission Spectrophotometry [16] The rate of uptake of aluminum into the brain was calculated according to the equation:

$$r = \frac{B_1 - B_0}{t}$$

where

r=rate of uptake

$B_1$=mean brain level of aluminum for group 1 upon sacrifice

$B_0$=mean control group brain level of aluminum upon sacrifice

t=the length of time which the rats had been fed aluminum (in days)

*Behavioral tests* All behavioral tests except the Shuttle-box test were administered the day before $AlCl_3$ $6H_2O$ dosing was commenced, and two and four weeks after the beginning of dosing On the days of testing, the morning dosing of aluminum was eliminated The Open Field and Roto-rod tests were given on the same day, whereas the Electroretinogram test was administered the following morning. The Shuttle-box test was administered only once, on the afternoon of the day before sacrifice

*Open field maze* The rats were transferred directly from their home cages to the test apparatus in a 15×10×15 cm handling box which had a sliding floor The box was placed in the center of the open field and the sliding floor removed After a two minute acclimation period the handling box was raised from an adjoining room by a pulley The following data were recorded over a ten minute period

1. Start time the time taken for the rat to initially move from the innermost circle after the handling box was raised
2. Radial movement: the number of sectors entered in each of circles two, three, and four (the outer circle)
3. Time, Circle 4: the total time spent in the sectors of the outermost circle
4. Side-center crossings the number of times the rat moved between individual circles
5. Inactivity the total duration of periods in which the rat remained in one sector longer than three seconds.
6. Defecation: the number of fecal boluses deposited during the 10 minute testing period
7. Total distance total distance traveled in the open field maze was calculated using the equation

$$D = \sum_{i=1}^{4} d_i s_i + \sum_{j=1}^{4} d_j c_j$$

where

D=total distance

$d_i$=the arc length connecting the midpoints of the straight sides of a sector in a given circle

$s_i$=the number of sectors crossed circumferentially in a given circle

$d_j$=the distance connecting the midpoints of the curved sides of a sector in a given circle

$c_i$=the number of sectors crossed radially in a given circle

*Roto-rod* The roto-rod test was administered after testing for open field behavior. The rats were transferred to individual cages for the duration of the testing to facilitate handling

On the first test date, the rats were trained on the rotating rod they were repeatedly placed on the rod until they had accumulated one minute of walking time. After training, actual testing began, with the stipulation that a trial last a minimum of five sec and a maximum of 15 min. The length of time until the rat fell from the rotating rod into the catch net was recorded All animals were tested sequentially before starting a new trial, and three trials were averaged

*Electroretinogram* After a minimum of five hr dark adaptation, each rat was anesthetized with sodium pentobarbitol (Nembutal, 40 mg/kg) The portion of the pinna occluding the auditory meatus was treated with Lidocaine (Xylocaine-HCl) and then clipped; ear pins were inserted, and the rat was secured in a stereotaxic apparatus Atropine was administered topically to the cornea to dilate the pupil and the anesthetic Proparicaine HCl (0 5%) (Opthaine) was applied to both eyes. The upper eyelids were then sutured and held open with weights. The recording electrode was placed in one eye and a reference electrode was placed in the other eye The flicker rate was increased until the response was indistinguishable from the 60 cycle background hum Two additional measures were immediately taken and the average of the three was recorded After this, the animal was identified and placed in a recovery cage. Three such trials were made in the course of the study

*Shuttle-box* Prior to the first trial, each rat was placed in the white portion of the box for 45 sec The rat was then placed in the black end of the box with the base of its tail touching the end wall. The partition separating the ends of the box was raised After 10 sec, a shock was delivered to the grid under the black portion of the box for 15 sec. If the rat remained in the black section of the box he was then placed in the white half for 45 sec with the partition between the halves of the box closed If the base of the rat's tail had crossed into the white end of the box before or during the administration of the shock, the partition was immediately lowered and the rat was left in the white end for 45 sec

The rats were tested for 15 trials in a rotation of six rats The total time which the rat remained in the black portion of the box was recorded, as was the number of trials undertaken before the rat crossed to the white portion of the box before being shocked

## RESULTS

### *Human Study*

To perform statistical analyses on the data obtained from the human subjects, groups were formed on the basis of serum aluminum level after all testing had been completed The 93 subjects were rank-ordered by serum aluminum level, the 25 highest were chosen as the "high aluminum" group, and the 25 lowest were selected as the "low aluminum" group The mean serum aluminum level for the high group was 504 ng/ml, which was significantly greater than the low group mean of 387 ng/ml ($p<0$ 001 by Student's $t$-test). The subject's verbal reports of antacid consumption did not correlate with actual serum aluminum levels. Comparisons of age, sex, race, urban versus rural background, education level, disease, or medications taken other than antacids yielded no significant differences between the two groups (throughout this report, "not significant" will imply $p>0.05$)

Of the tests administered to the human subjects, three failed to produce significant differences between the groups the Digit Span, Trials A, and Block Design tests. Each of the other tests successfully differentiated between the groups the "low aluminum" group performed significantly better on the Digit Symbol, Trails B, Serial Sevens—Pauses and Serial Sevens—Errors tests, and demonstrated a lower sensitivity to flicker, than did the "high aluminum" group. Results of these tests are summarized in Table 1 Complete data was not obtained from all subjects. This is reflected in the varying group numbers listed in Table 1.

### *Rat Study–Group 1*

Drawing baseline blood samples contributed to the high rate of rat mortality in this group (see Fig. 1). (The dose levels included on Figures 1 to 5 refer to the brain levels listed in Table 2 Those figures which list "C" as the first point refer to results based on dose groups, whereas those which list "VL" refer to comparison groups.) However, enough rats survived to determine that there were no significant differences between groups with respect to baseline serum aluminum level. For the purpose of comparing behavioral test data for these rats, the animals were divided into four groups by brain aluminum level Therefore, terminal brain and serum levels of aluminum are shown both by dose and by analysis group in Table 2 Correlations were found between terminal serum aluminum levels and dose ($r=0$ 581,

TABLE 1

SIGNIFICANT HUMAN TEST RESULTS

| Test | $\bar{y} \pm$ SE | $r_i$ | $p$ |
|---|---|---|---|
| *†Digit Symbol | 15 05 ± 0 579 points | 21 | <0 05 |
| | 13 48 ± 0 600 | 21 | |
| ‡Serial Sevens—Pauses | 0 43 ± 0 173 | 14 | <0 01 |
| | 2 57 ± 0 761 | 14 | |
| §Serial Sevens—Errors | 1 375 ± 0 301 | 16 | <0 01 |
| | 2 500 ± 0 652 | 15 | |
| †Critical Flicker Frequency | 34 80 ± 0 762 Hz | 24 | <0 01 |
| | 37 16 ± 0 616 | 25 | |
| †Trails B | 107 75 ± 8 390 sec | 20 | <0 05 |
| | 125 00 ± 8 959 | 20 | |

*Mean score for "low $Al^{+3}$" group given first in each pair

†Analysis by Student's $t$-test

‡Analysis by Mann-Whitney U-test

§Analysis by Lohrding's Test of Two Means

$r_i$=the number of subjects per group

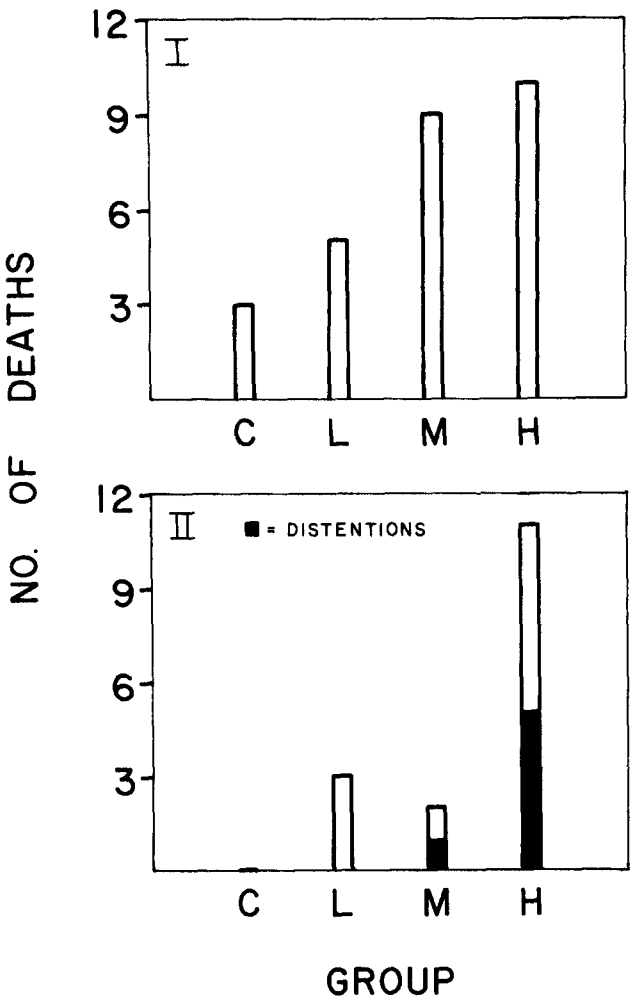


FIG 1 Rat mortality rates prior to sacrifice in studies 1 and 2, by dose groups The term "distentions" refers to the number of rats from Group 2 which died and in which distended stomachs were discovered upon autopsy Distentions were not recorded for Group 1 rats

TABLE 2
TERMINAL BRAIN AND SERUM ALUMINUM LEVELS

| Test | | $\bar{y} \pm SE$ | $r_i$‡ | $p$ |
|---|---|---|---|---|
| Group 1 | | | | |
| *Brain $Al^{+3}$ (by dose) | control | 690 ± 18 ng/g† | 9 | <0 001 |
| | low | 794 ± 14 | 9 | |
| | medium | 929 ± 24 | 7 | |
| | high | 921 ± 27 | 7 | |
| Brain $Al^{+3}$ (by comparison group) | very low | 653 ± 17 ng/g | 6 | <0 001 |
| | low | 758 ± 5 | 6 | |
| | medium | 863 ± 16 | 6 | |
| | high | 967 ± 9 | 6 | |
| Serum $Al^{+3}$ (by dose) | control | 725 ± 19 ng/ml | 10 | <0 005 |
| | low | 734 ± 29 | 7 | |
| | medium | 833 ± 47 | 4 | |
| | high | 850 ± 26 | 7 | |
| Serum $Al^{+3}$ (by comparison group) | very low | 717 ± 31 ng/ml | 6 | <0 005 |
| | low | 728 ± 30 | 6 | |
| | medium | 786 ± 23 | 5 | |
| | high | 917 ± 7 | 3 | |
| Group 2 | | | | |
| Brain $Al^{+3}$ (by dose) | control | 688 ± 16 ng/g | 11 | <0 005 |
| | low | 700 ± 14 | 11 | |
| | medium | 818 ± 22 | 9 | |
| | high | 820 ± 31 | 7 | |
| Brain $Al^{+3}$ (by comparison group) | very low | 645 ± 4 ng/g | 10 | <0 005 |
| | low | 708 ± 4 | 10 | |
| | medium | 768 ± 5 | 10 | |
| | high | 888 ± 7 | 10 | |

*Analysis by Bonferroni $t$-test (one-way ANOVA)
†Brain aluminum values correspond to wet weight of tissue
‡$r_i$=the number of subjects per group

$p<0$ 001), between serum and brain aluminum levels ($r=0$ 628, $p<0.001$), and between brain level and dose ($r=0.829$, $p<0$ 001).

Analysis of the behavioral test data for Group 1 showed no significant differences between groups with respect to baseline or final administration of each test, using one-way analysis of variance $t$-tests.

*Rat Study–Group 2*

Baseline serum aluminum levels were not determined for Group 2 due to the high rate of mortality resulting from this procedure in Group 1. However, there were also deaths in the Group 2 test population (see Fig. 1). As the only abnormal finding upon autopsy of Group 1 rats dying for reasons other than heart puncture was the presence of distended stomachs, distensions were noted for Group 2 rats which died Terminal brain aluminum levels are shown by dose and analysis group in Table 2. In forming the analysis groups, 17 shifts were made from one dose group to another. These are evenly distributed between control and low movements, and medium and high movements. The eight Amphojel-fed rats included in the analysis groups were also distributed relatively evenly across groups. Serum aluminum levels were not significantly different across groups when compared by dose or by analysis group. Furthermore, terminal serum aluminum levels were not correlated with dose or with brain aluminum levels However, brain aluminum levels were found to be correlated with dose ($r=0$ 649, $p<0$ 001).

Analysis of baseline data for all behavioral tests showed no significant differences between analysis groups for any test except the ERG (refer to Discussion)

Analysis of final test data using one-way ANOVA Bonferroni $t$-tests showed no significant differences between groups on the majority of the tests, including blood hematocrit and weight gain over the testing period. However, significant differences were seen with respect to the roto-rod ($p<0$ 001, see Fig 3), and total distance traveled in

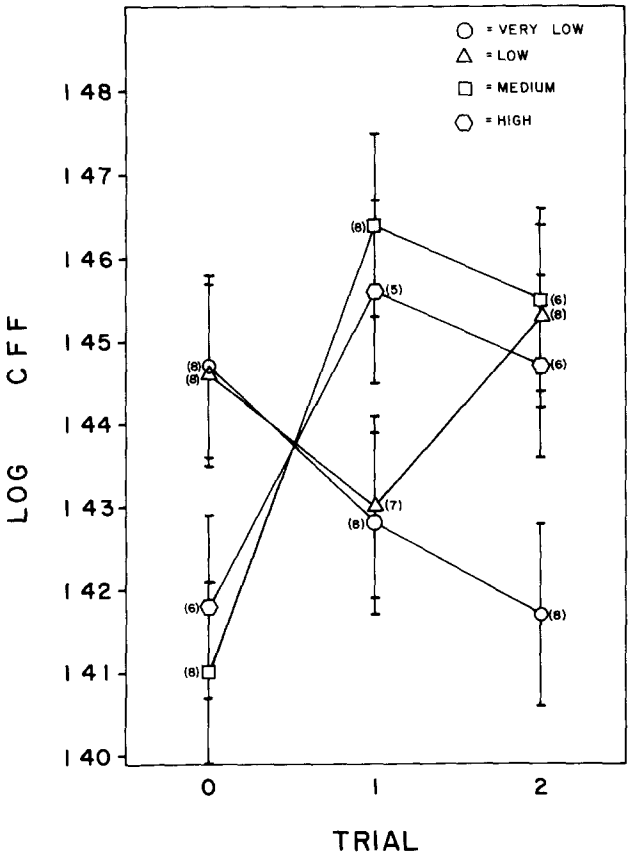


FIG 2 Log of critical flicker frequency (CFF) over time, by analysis group (mean ± SE) The interval between trials is two weeks The numbers in parentheses refer to the number of rats per analysis group for a given trial

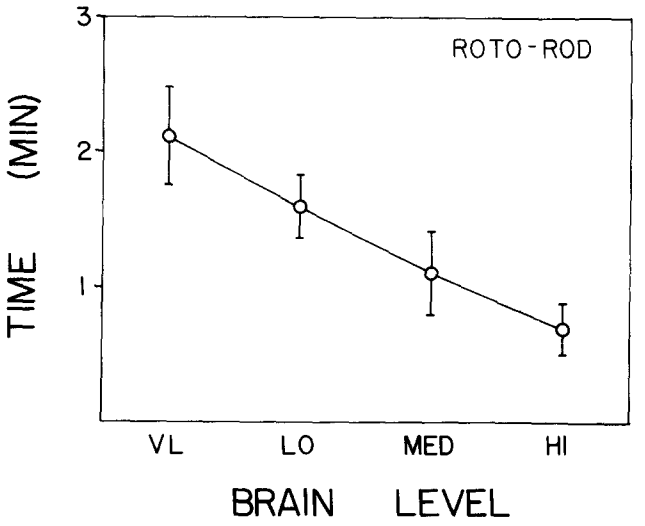


FIG 3 The amount of time roto-rod activity was maintained in the last test before sacrifice, by analysis group (mean ± SE)

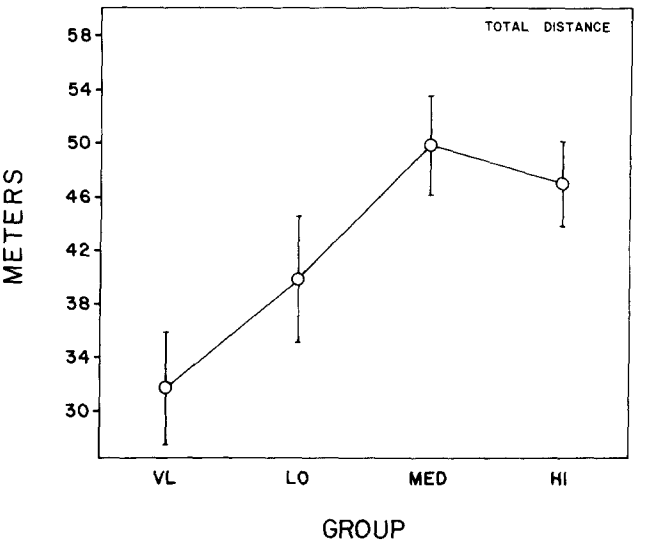


FIG 4 Total distance traveled in the open field maze in the last test before sacrifice, by analysis group (mean ± SE)

the open field maze ($p<0$ 025, see Fig 4). Differences were also seen with respect to the ERG test between the very low comparison group and the other three comparison groups ($p<0$ 05, see the final trial of Fig. 2) Further analysis yielded a figure showing trends over time for the comparison groups (The light-housed animals were excluded from these groups due to lack of data This resulted in basically no change in the mean brain aluminum concentration of the comparison groups.) This graph clearly demonstrates the existence of unexpected significant differences between the very low/low and the medium/high group performances in the baseline and first trials

Possible explanations for the baseline differences in the flicker fusion rates of the Group 2 rats were considered One possibility discussed was the age of the rats at the time of initial testing· all rats were 60 days old when received from the supplier, but baseline testing (and the commencement of $AlCl_3$ administration) was staggered over a period of 2 weeks. The mean performance of the rats tested on each day was compared and no differences were found In addition, no differences were found between the performance of the rats tested during the first weeks as compared with those tested during the second week This pehnomenon was therefore considered to be a statistical anomaly

The mean brain level for the 15 rats which were intubated with Wyeth-Amphojel was 761 ng/g, which was not significantly different from the brain aluminum levels of the medium and high dose $AlCl_3$-fed rats Likewise, their mean serum aluminum level, 702 ng/ml was not different from the serum aluminum levels of all the $AlCl_3$-fed rats Also, levels of aluminum in serum and brain were here found to be correlated ($r=0$ 728, $p<0$ 001). Their performance on the behavioral tests, after four weeks of aluminum ingestion, did not differ from that of the $AlCl_3$-fed rats of comparable brain aluminum level (It may be unwise to directly compare the test results for the Amphojel-fed rats with those of the other test animals as the groups were housed under different lighting conditions However, the lack of significant differences on the ERG test between Amphojel-fed animals and $AlCl_3$-fed animals of comparable brain aluminum levels, despite these lighting differences, seems to support the comparability of the results of the other tests ) There were no deaths in the Amphojel-fed group during the four weeks of the study

TABLE 3
RATES OF $AL^{+3}$ UPTAKE INTO THE BRAIN

| | Rates | |
|---|---|---|
| | By Dose | By Comparison Group |
| Group 1 (t=21d) | | |
| Control | 0 ng/g/day* | 0 ng/g/day |
| Low | 4 9 | 3 2 |
| Medium | 11 4 | 8 2 |
| High | 11 0 | 13 2 |
| Group 2 (t=26d) | | |
| Control | 0 | 0 |
| Low | 0 46 | 0 77 |
| Medium | 5 00 | 3 08 |
| High | 5 04 | 7 69 |
| High (t=13d) | 5.85 | — |
| Amphojel (t=26d) | 2 81 | — |

*These values cannot be considered exactly zero as even the control rats were receiving some aluminum in their feed and drinking water

Similarly, there were no deaths among the rats fed NaCl of varying osmolality and pH. It is therefore probable that neither of these two parameters was the sole cause of rat mortality in Group 1.

Finally, analysis of the rates of uptake of aluminum into the brain for all groups indicates that such uptake is dependent upon the dose administered and not upon the duration of administration (see Table 3) It is noteworthy that although individual rates of aluminum uptake into the brains of Amphojel-fed rats varied considerably, the mean rate was intermediate between the mean rates for the low and medium $AlCl_3$ dose groups

## DISCUSSION

The results obtained from the human subject data suggest that high serum levels of aluminum are associated with impaired complex visuo-motor coordination, poor long-term memory, and increased sensitivity to flicker. Some of these findings were anticipated on the basis of previous animal research, as noted in the introduction. Visuo-spatial organization, simple visuo-motor coordination, and short-term memory do not appear to be correlated with serum aluminum level This might be biased by the difficulty of the tests of the latter parameters, however the Block Design test was a difficult task for most of the subjects, resulting in a preponderance of low scores, conversely, the Trails A test was relatively simple, with the result that most of the subjects achieved high scores Neither test could therefore be regarded as a good discriminator

It is of considerable interest that serum aluminum levels did not appear to correlate closely with reported ingestion of aluminum-containing antacids. While it is possible that the verbal reports of antacid consumption are not accurate, it may well be that there are significant alternative sources of aluminum which have confounded the correlation.

It was found that aluminum, ingested orally in the form of $AlCl_3$ or $Al(OH)_3$, is absorbed by rats with ostensibly normal renal function and deposited in the brain. This supports previous work [2] contradicting the traditional belief that orally ingested aluminum is almost totally unabsorbed from the gut [13] Since serum levels of aluminum were correlated with brain levels of aluminum for Group 1 and Amphojel-fed rats but not correlated for Group 2 rats, there is some uncertainty as to whether serum aluminum concentration is a reliable indicator of brain aluminum concentration However, the fact that there were significant positive correlations between serum and brain aluminum levels in two of the three groups tends to support the use of serum concentrations of aluminum for the purpose of comparing the behavioral test data of the human subjects in this study

While it could be argued that the results of the behavioral tests might be biased by general illness imparted by stomach-loading aluminum chloride, the lack of significant differences between analysis groups with respect to weight gain, hematocrit at sacrifice, and the majority of the measures of general activity indicate that this is not so It might also be suggested that these animals suffered from phosphate depletion, the usual explanation for symptoms associated with aluminum ingestion However, gross indicators of this condition, such as low weight gain, rough and greasy coats, lack of movement, walking with a "crippled gait," and watery eyes [11] were not evident in our test population

The results from the shock-avoidance task suggest that learning ability is not impaired by aluminum However, the one-way shock avoidance measure may have been too simple a task to make discriminations between test groups

The results of the open field maze suggest raised excitability due to aluminum intoxication the animals with the higher brain aluminum levels covered a greater distance than did the lowest brain aluminum analysis group while starting at the same time and spending the same amount of time in the outer sectors This means that the animals with higher aluminum levels were traveling faster It would be difficult, therefore, to conclude that gross motor ability is directly impaired by aluminum ingestion Assuming that it is not, the results of the roto-rod test are also compatible with an excitability model greater distractability, an oft-mentioned symptom of hyperactivity in children, could easily account for the decreased ability of the higher aluminum level rats to maintain roto-rod activity

Analysis of the electroretinogram results from the final test date revealed significant differences in performance between the very low analysis group and the other three analysis groups. However, further analysis indicated that for this test the baseline performance of the medium and high analysis groups differed significantly from that of the low and very low analysis groups. The discrepancies between the very low/low and medium/high group performances for both the baseline and first test are significant at $p<0$ 05

We are proposing a model to explain the results obtained from the ERG test Figure 5 is a hypothetical extension of obtained data graphically depicting the model. This model supposes that aluminum accumulates to a critical level, at which point it causes CFF to increase to an age-determined maximum Once the ceiling value is attained, the CFF begins to decrease in accordance with the age trend shown by the very low group As can be seen in Figure 5, the low brain aluminum comparison group demonstrates this hypothesis very well, as it shifts direction with increasing aluminum concentration Finally, the essentially parallel declines of the high, medium, and very low groups after Trial 1 are also noteworthy

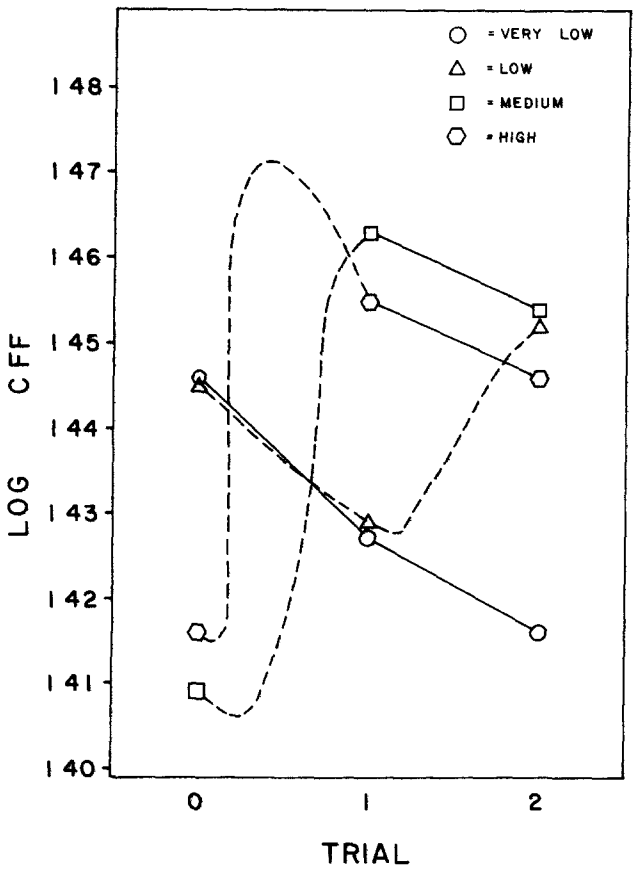


FIG 5 A hypothetical extension of the data obtained from the last electroretinogram test before sacrifice, by analysis group. Data points and solid lines are from Fig 2, whereas dashed lines represent hypothetical continuations between data points

The fact that both rat and human subjects with high aluminum levels demonstrated increased sensitivity to flicker strongly indicates that this parameter is affected by aluminum ingestion. In view of this, and previous research indicating that injections of $AlCl_3$ result in neurofibrillary degeneration [10], it appears that aluminum impairs aspects of central nervous system function.

In conclusion, a model supposing that aluminum increases excitability in rats will account for the results of the animal portion of this study The human subject results support the work performed on cats and other small mammals in which neurofibrillary degeneration was induced. It is especially interesting that both the human subjects with high serum concentrations of aluminum, and the rats which had been fed aluminum demonstrated an increased sensitivity to flicker.

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## REFERENCES

1 Alfrey, A C., G R LeGendre and W D Kaehny The dialysis encephalopathy syndrome—possible aluminum intoxication *New Engl J Med* **294**: 184–188, 1976
2 Berlyne, G M., J Ben An, E. Knopf, R Yagel, G Weinberger and G M. Danovitch Aluminum toxicity in rats *Lancet* **1**: 564–567, 1972
3 Brown, J L Flicker and intermittent stimulation In *Vision and Visual Perception*, edited by C H Graham New York John Wiley and Sons, 1965
4 Cicerone, C M. Cones survive rods in the light-damaged eye of the albino rat *Science* **194**: 1183–1185, 1976
5 Cornsweet, T. N The staircase method in psychophysics *Am J Psychol.* **75**: 485–491, 1962
6 Crapper, D R and A. J. Dalton. Aluminum induced neurofibrillary degeneration, brain electrical activity and alterations in acquisition and retention. *Physiol Behav* **10**: 935–945, 1973
7 Crapper, D R and A J Dalton Alterations in short-term retention, conditioned avoidance response acquisition and motivation following aluminum induced neurofibrillary degeneration *Physiol Behav* **10**: 925–933, 1973
8. Crapper, D R , S S Krishnan and A. J Dalton Brain aluminum distributed in Alzheimer's disease and experimental neurofibrillary degeneration. *Science* **180**: 511–513, 1973
9 Crapper, D. R , S S Krishnan and S Quittkat Aluminum, neurofibrillary degeneration and Alzheimer's disease *Brain* **99**: 67–80, 1976
10 Crapper, D R and G. J. Tomko Neuronal correlates of an encephalopathy associated with aluminum neurofibrillary degeneration *Brain Res* **97**: 253–264, 1975
11 Day, H G. and E V McCollum Mineral metabolism, growth, and symptomology of rats on a diet extremely deficient in phosphorus *J biol Chem* **130**: 269–283, 1939
12 Dialysis dementia aluminum again? *Lancet.* **1**: 349, 1976.
13 Falconer, M W , H. R. Patterson and E. A Gustafson. *Current Drug Handbook.* Philadelphia· W B Saunders, 1976–1978
14 Kaehny, W M., A. P Hegg and A C. Alfrey Gastrointestinal absorption from aluminum-containing antacids *New Engl J Med* **296**: 1389–1390, 1977
15 Lezak, M D. *Neuropsychological Assessment.* New York Oxford University Press, 1976.
16 Mayor, G. H , J A Kaiser, D Makdani and P K Ku Aluminum absorption and distribution. effect of parathyroid hormone *Science* **197**: 1187–1189, 1977
17 Overman, S R Behavioral effects of symptomatic developmental plumbism in rats Unpublished doctoral dissertation, Michigan State University, 1976
18. Price, E O and W. Huck. Open field behavior of wild and domestic Norway rats *Anim Learn Behav* **4**: 125–130, 1976
19 Reitan, R M The relation of the trailmaking test to organic brain damage. *J consult Psychol* **19**: 393–394, 1955.
20 Wechsler, D. *The Measurement of Adult Intelligence* Baltimore Williams and Wilkins, 1955.