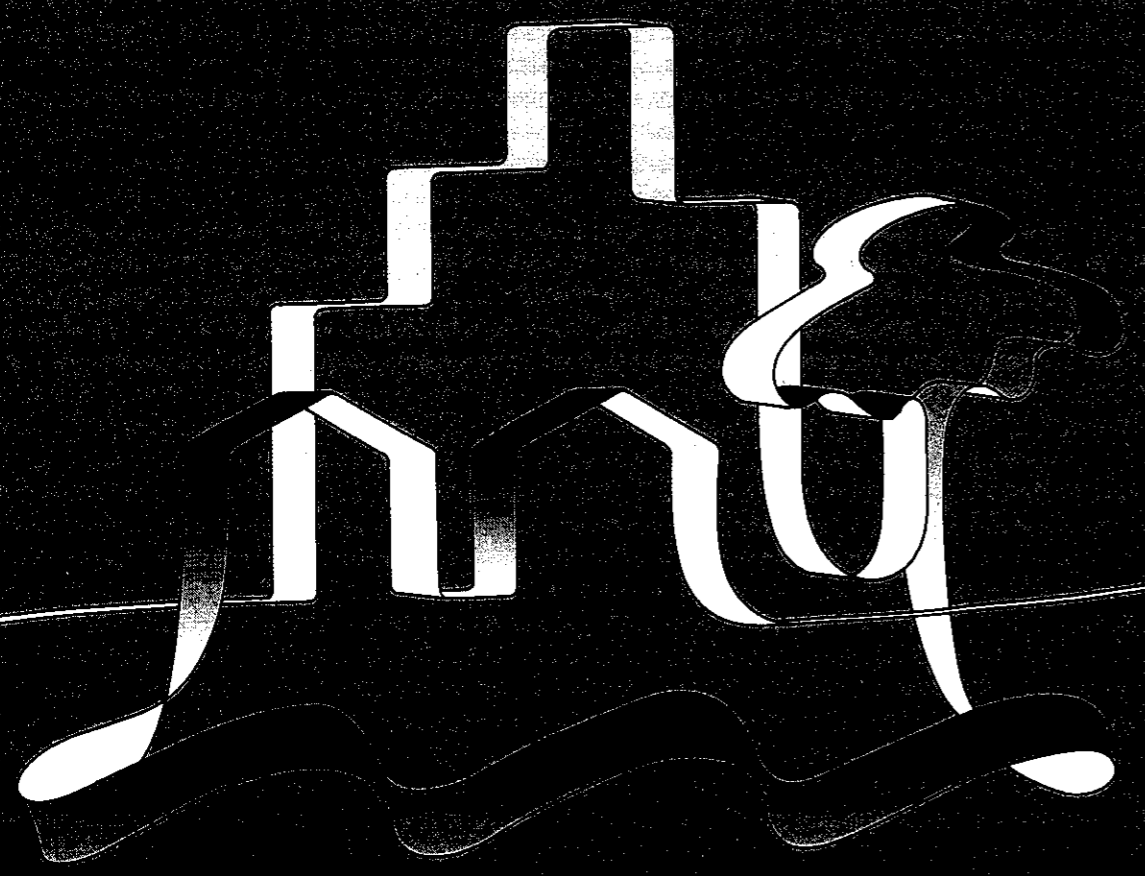


Bioavailability of Vitamins from Drinking Water Co-exposure with Foods and Beverages



Journal of the Food and Nutrition Society

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Bioavailability of Aluminium from Drinking Water: Co-exposure with Foods and Beverages

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FOREWORD

This report is based on UWRAA Research Project No WS-58: 'Bioavailability of aluminium in drinking water' which was undertaken during the period July 1992 - June 1994.

The project was undertaken by the Australian Institute for Biomedical Research Ltd with Dr Judie Walton as the Principal Researcher. The consultancy was administered by the Sydney Water Board on behalf of the Urban Water Research Association of Australia, which funded the project.

SYNOPSIS

Alum (aluminium sulphate) is used in the clarification process of raw water for drinking. A recent study using Sydney drinking water has shown that after alum treatment it contained a higher proportion of soluble to insoluble aluminium species. Soluble forms of aluminium are bioavailable and toxic. They are readily absorbed from the digestive tract into the bloodstream and taken up by bone, brain and other soft tissues.

At high blood levels, aluminium causes acute dementia (dialysis dementia) in some renal failure patients. Epidemiological studies have linked aluminium content in drinking water to the incidence of Alzheimer's disease, a dementia which usually develops over a longer time course.

The main information known about aluminium bioavailability is that citric acid from lemon juice combines with aqueous aluminium to form aluminium citrate. This is much more readily absorbed than aqueous aluminium alone. The present research studied whether other nutrients affect aluminium bioavailability from drinking water. Laboratory rats were used to establish a "relative aluminium bioavailability" database for each test diet. Changes in serum and urinary aluminium concentrations were measured as an index of aluminium absorption/bioavailability.

This research showed that diet is a prominent determinant of aluminium bioavailability from drinking water. Orange juice, coffee and wine significantly increase the amount of aluminium absorbed from water. Tea, beer, butter, and apple have no effect while beef and Vita Wheat biscuits tend to decrease aluminium absorption. For each test diet a subpopulation of rats had higher aluminium bioavailability than others and were more at risk. This finding is consistent with previous research on humans (Taylor et al., 1992; Harrington et al., 1994).

From this study and other available data, it becomes clear that aluminium is absorbed through the intestine from alum-treated drinking water. However, almost all the known facts relate to rats. Further research is urgently required to ascertain if absorption occurs to the same degree in humans. If this proves to be the case, then the small daily amount of aluminium that gains access to the body must be critically analysed to find if it produces accumulated damage to brain tissues, particularly in the elderly.

BIOAVAILABILITY OF ALUMINIUM FROM DRINKING WATER:
CO-EXPOSURE WITH FOODS AND BEVERAGES

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1. INTRODUCTION

1.1 Aluminium in Water, Foods, and Beverages

Alum is used in the treatment of municipal water supplies to serve as a coagulant in the water clarification process. The total aluminium content of alum-treated drinking water usually varies between 50-200 micrograms per litre ($\mu\text{g/L}$) although under certain conditions this range is greatly exceeded. In two surveys, alum treatment of raw water sources raised their total aluminium content despite removing particulate aluminium (Hiller et al., 1984; Schenk et al., 1989). More specifically, this treatment has been found to increase the proportion of soluble or bioavailable aluminium relative to insoluble species (Tran et al., 1993).

The residual aluminium concentrations in finished waters are a function of the aluminium levels in the source water, the dosing of aluminium coagulant, and the efficiency of filtration of the aluminium precipitate or "floc". Where residual concentrations are high, aluminium deposition may occur in the distribution system. Despite the widespread practice of adding alum to drinking water, with the exception of one report (Allen & Fontenat, 1984), very little biological research has specifically focussed on alum.

Foods and beverages all naturally contain some amount of aluminium. The amount depends on their chemical composition as well as how and where they are produced. Natural foods contain, on the average, less than 0.5 mg aluminium per gram wet weight (Greger et al., 1985). In some food and beverages it is strongly complexed and in others less so. For example, aluminium naturally found in tea leaves is strongly bound by polyphenols so it is poorly absorbed across the gastrointestinal tract (GIT) lining into the bloodstream.

Aluminium generally enters the food chain from acid soils which have greater amounts of aluminium in soluble form. Alum-treated irrigation water, acid rain, and acid-producing fertilisers contribute to this situation. Due to its toxicity, high soil concentration of soluble aluminium is an important limiting factor to plant growth (Foy, 1974).

In addition to that which occurs naturally, many commercially-prepared foods have aluminium compounds added to them to endow them with certain properties (Pennington, 1987). For example, aluminium ammonium sulphate is added to pickles as a hardening agent, aluminosilicate to salt to make it free-pouring, sodium aluminium phosphate to processed cheeses as an emulsifier. Experiments with dogs showed that a large percentage of

the aluminium in bread made with alum-based baking powder was soluble in gastric juice (Jones, 1938) and thus readily absorbed into the blood (Underhill and Peterman, 1929a).

Due to the universality of aluminium in food, water, beverages, and some medications, all people absorb some elemental aluminium from their diet. The human bloodstream is estimated to contain, on the average, approximately $6 \mu\text{g}$ aluminium/L (Kaehny et al., 1977) but this amount is variable between individuals.

1.2 Aluminium Ingestion

In alum-treated water, aluminium sulphate and free aluminium are present at low pH. When the pH is raised there is a shift towards the formation of aluminium hydroxide which is virtually insoluble in the range of pH 6.3 to 7.8. If the pH is raised above 7.8, negatively-charged aluminium hydroxy ions form and these may also be soluble.

The various salts of aluminium behave differently in the body than they do in non-ingested water. Until the 1970s, the gastrointestinal tract lining was thought to be an impervious barrier to aluminium uptake in healthy people and animals. However, in 1977, Kaehny et al. clearly demonstrated that the GIT lining of normal subjects, though a formidable barrier to the entry of aluminium, is not impervious.

Aluminium metabolism is extremely complex and it has aspects which are not yet understood. When drinking water is taken into the human stomach it is acidified in the gastric acid (hydrochloric acid) which has a pH of about 2. Hence, some of the less soluble aluminium in water can be readily solubilised in the stomach and made available for absorption. Free aluminium ions also react with gastric acid to produce aluminium chloride.

As water moves from the stomach into the small intestine its pH rises through neutrality and then to an alkaline pH of around 8. Intestinal conditions tend to inhibit the precipitation of aluminium at a pH where it would normally precipitate if contained in water alone (Partridge et al., 1989).

In a normal meal, a variety of foods are consumed. The mixture of foods, beverages, and their breakdown products at various stages of digestion in the GIT is extremely complex and so are the chemical interactions which can occur between them.

Digestion is the process by which the living body breaks food down into simpler chemical compounds which can be absorbed into the bloodstream and delivered as nutrients to cells. The digestive process is partly mechanical (e.g., chewing in the mouth and churning movements in the stomach). It is partly chemical through the action of the digestive enzymes. These enzymes are secreted into the upper part of the small intestine (the duodenum) where they continue to break down and dissolve the nutrients.

Dissolved nutrients, together with a proportion of the ingested aluminium, are picked up by the cellular lining of the GIT and absorbed across it into the blood and lymph vessels within the GIT. The undigested food residues continue down the tract in preparation for their removal from the body as faeces. During this time, water and ions of aluminium and other inorganic elements are progressively absorbed from the GIT tract into the blood compartment, causing the faecal content to become more solid.

In order for aluminium to be absorbed, it needs either to be soluble or at least held in a form that can be taken up by cells of the gastrointestinal lining. Aluminium is known to bind, at least at an acid pH, to mucus glycoproteins associated with the GIT lining (Crowther and Marriott, 1984). Thus, direct interaction of the gastrointestinal lining with monomeric aluminium, or stabilisation of a polymeric or even colloidal species are all possible. Most aluminium appears to be absorbed across the gastrointestinal lining of the duodenum and jejunum (the first two sub-divisions of the small intestine) by passive diffusion between cells.

Pharmacokinetic estimates of aluminium absorption across the digestive tract lining have varied from 0.001% to 10% (Wilhelm et al., 1990). The amount absorbed depends on the form of aluminium ingested, its concentration, digestive tract pH, other nutrients in the GIT, genetic characteristics and variations between different investigators and the experimental protocols they use. For example, the oral bioavailability of aluminium in renally intact rabbits ranged from 0.3 to 2.2% with aluminium borate < glycinate < hydroxide < chloride < sucralfate < lactate < nitrate < citrate (Yokel and McNamara, 1988). Unfortunately, comparable data for determining aluminium sulphate (alum) absorption was not included. However, as these measurements were all based on ingestion of milligram concentrations of aluminium, they may be *quantitatively* irrelevant to the actual absorption levels that occur when trace amounts of aluminium are ingested in water.

The present experiment was designed to compare *relative* aluminium bioavailability. That is, to indicate possible chemical reactions between alum in water and the ingested foods or

beverages which may affect its amount of uptake into the bloodstream and its bioavailability to tissues and cells.

Soluble aluminium has long been known to be toxic to living tissues, particularly affecting brain, bone and blood cells. One hundred micrograms (μg) aluminium per litre of blood (0.1 ppm) greatly increases the risk of toxicity. When directly applied to the brain of experimental animals, soluble aluminium produced intense epileptic seizures which resulted in death within a few hours (Kopeloff et al., 1942). This did not occur with equivalent concentrations of copper or silver.

Most investigations related to acute aluminium toxicity in humans has focused on kidney failure patients. Other patient groups already identified as being susceptible to acute aluminium toxicity also have impaired ability to excrete aluminium from the bloodstream. The latter group includes people on long-term intravenous feeding, premature newborns, and liver failure patients. Very elderly people may be at increased risk for aluminium intoxication in view of the 50% decline in kidney function which occurs by age 85 (Hamburger and Crosier, 1979) combined with a long exposure period.

Some food researchers have expressed doubt as to whether the aluminium in drinking water makes a significant contribution to the total body aluminium burden (Greger and Baier, 1983; Pennington and Jones, 1989). They estimate that only 2% of the total daily aluminium ingested comes from drinking water. Of this, the daily amount of aluminium actually absorbed daily from drinking water has been estimated at 4 μg daily (Powell and Thompson, 1993). This is based on an adult drinking 2 litres of water containing 0.2 mg aluminium per litre and absorbing 1% of the ingested amount.

However, this formula is at best a guess. Firstly, it assumes that aluminium absorption is proportional to the amount ingested. Moreover, it does not take into account the various conditions under which people consume water and, in particular, how other digestive tract constituents interact with drinking water aluminium to affect its absorption.

Martyn et al. (1989) have suggested that drinking water aluminium may be more bioavailable than food aluminium because much of it is soluble or can be readily solubilised. This contrasts with the forms of aluminium naturally contained in foods which often tend to be bound. Other considerations are that 1) foods buffer acid in the stomach, raising its pH to a level where aluminium solubility should be reduced; and 2) with the exception of aluminium food additives, most foods are probably poor sources of soluble aluminium (Powell and

Thompson, 1993). Experimental evidence for this comes from a study in which aluminium in drinking water, but not in the food, influenced in rats brain bipterin metabolism--a feature affected in Alzheimer's disease (Armstrong et al., 1992).

The effects, if any, of lifetime, chronic exposure to aluminium at drinking water levels, are less well-known. Seven epidemiological studies have reported an increased incidence of Alzheimer's disease (AD) in regions with relatively high aluminium levels in their drinking water supply (Crapper-McLachlin et al., 1991). However, chronic toxicity is much more subtle to trace than acute (short-term, high exposure level) toxicity. To evaluate whether AD results from chronic exposure to drinking water aluminium, much more information is required about aluminium metabolism in young and old individuals than is currently known.

1.3 Plasma and Urinary Aluminium

The proportion of ingested aluminium that is absorbed enters the blood circulation and some may also enter the lymphatic circulation (Underhill and Peterman, 1929a). Aluminium is transported in blood by a variety of plasma components. The aluminium species in plasma can be separated into a number of fractions including: 1) an ultrafiltrable, probably unbound fraction comprising approximately 10% of plasma aluminium, 2) small molecule plasma binders (citrate, fluoride, phosphate, bicarbonate, and low molecular weight proteins); 3) albumin; 4) transferrin; and 5) other larger molecular weight proteins (King et al., 1979; Martin, 1986). Transferrin is thought to be the most important plasma aluminium carrier. It normally transports iron in blood but has a second binding site often occupied by aluminium (Huebers and Finch, 1987).

Total aluminium content of the blood of the same normal person may vary from time to time during narrow limits (Underhill and Peterman, 1929b). Plasma (serum) aluminium levels also vary from person to person, due both to their dietary choice and their metabolic characteristics. Some people consume a diet relatively rich in aluminium, particularly if they depend on prepared foods containing aluminium additives. Others may have higher than normal aluminium blood levels because of their inherited metabolic (absorption and excretion) patterns.

Unknown amounts of the bound and unbound plasma aluminium are taken up by tissues, particularly in areas rich in transferrin receptors (Edwardson et al., 1993a). Little is known about the disposition of aluminium once it has entered the cells of a tissue --whether it can be

returned to the blood or whether it is there to stay. Aluminium stored in bone over the life span may parallel the behaviour of calcium stored in bone. For example, in osteoporosis (age-related bone deterioration) calcium salts leave the bone and return to the circulation where they can be delivered to soft tissues. Given the same circumstances, aluminium salts accumulated in bone may also return to the circulation in old age.

When alum is ingested orally, absorption into the blood continues for some time so that aluminium half-life in the blood is longer than for intravenous injection (Yokel and McNamara, 1985). Unbound plasma aluminium not picked up by tissues tends to be excreted either into the urine or into the bile.

The main excretory organ for aluminium is the kidney and the amount excreted depends on largely on the form and the amount of aluminium that has been absorbed. Froment et al. (1989), who fed 35 mg/kg aluminium chloride to 250-380 g rats, reported finding 0.4% of the oral dose excreted in the urine. With 24 mg/kg aluminium citrate, this figure rose to 1.5% (74 $\mu\text{mol/L}$) which was 50 to 150 times more than aluminium hydroxide.

According to Burnatowska-Hledin (1985), less than 8.4% of plasma aluminium is ultrafilterable in the kidney and of this a significant fraction--as high as 85%--may be reabsorbed back into the bloodstream during its passage through the kidney tubules. That which is not reabsorbed passes into the urinary bladder and is then removed from the body.

Some aluminium taken up by the liver is secreted into the bile which then empties into the GIT. However, there it is once again available for reabsorption back into the bloodstream further down the digestive tract. To further complicate the picture, aluminium is also removed from the blood by other tissues and body fluids. Thus, in principle, plasma aluminium measurements for bioavailability estimates are necessarily low. This very complex scenario, and the state-of-the-art instrumentation currently available for routine aluminium measurement, make quantified studies of aluminium metabolism difficult to compare and interpret.

Ideally, these trace amounts would be best studied with the radioisotope aluminium-26 and accelerator mass spectrometry (AMS). However, the costliness of the isotope and the AMS measurements limit the number of samples that can be examined by that approach. The more practical solution is to elevate the aluminium dose to a pharmacological level which can be detected by GFAAS (sensitivity $>2.7 \mu\text{g/L}$). This is generally regarded as the most

expedient way to estimate aluminium absorption and has formed the basis of most aluminium experiments in humans and animals.

Hence, most calculations of aluminium bioavailability rely on GFAAS measurements of aluminium in plasma (serum) and urine taken either from humans or experimental animals dosed with higher than physiological aluminium levels. Full balance studies are impractical because the percentage of absorbed aluminium is so low that the smallest error in stool collection or measurement of aluminium in processed stool can give rise to a large error. Hence, it is more useful to focus on the amount absorbed than on full balance studies including the faecal content (Froment et al., 1989).

1.4 Animal-based Research

Most of what is known about human physiology has been based on experimental investigations of other mammals. Animal-based experiments are especially vital to aluminium research because of human ethics considerations, given that it is a neurotoxin. In general, for human nutritional studies, rats are a particularly good experimental model for three main reasons: (1) Both humans and rats are omnivorous feeders. (2) Their digestive tracts share structural similarities. (3) Their stomach is a single compartment in contrast to the rabbit stomach which is multi-compartmental. Moreover, their feeding can be more rigorously controlled than in human experiments.

Rats are also useful as a general model for lifetime exposure studies. They grow old in 2-3 years and show many of the same age changes that affect humans over 7-8 decades, such as memory loss, tumours, osteoporosis, and cataracts.

1.5 Previous Findings About Aluminium Bioavailability

The literature on aluminium bioavailability and how it is influenced by nutrition is relatively small. In this section, most of the extant literature pertaining to the effects of foods, beverages, and other dietary factors known to affect aluminium absorption and bioavailability is summarised.

1.5.1 Food acids

Computer simulations (Dayde and Berthon, 1990) predicted that aluminium absorption could be enhanced by malic, oxalic, succinic and tartaric acids which are normal dietary acids; i.e. products of food digestion. Lactate, pyruvate, albumin, and lactoferrin are generally present in intestinal secretions and have been hypothesised to prevent the precipitation of dietary aluminium, thereby enhancing its solubility and absorption during transit. Both citrate and maltol have been found to stimulate aluminium uptake (Domingo et al., 1991a).

The well-known enhancement of aluminium absorption by dietary citric acid, reported by Slanina et al. (1984), has been widely used as a tool to study aluminium uptake. Given as lemon juice, citric acid combines with aluminium in the stomach at acid pH (usually around pH 2.5-3). In healthy men, plasma aluminium levels rose from 5 micrograms aluminium/litre ($\mu\text{g/L}$) pre-treatment to 12 $\mu\text{g/L}$ following a dose of aluminium hydroxide. They increased further, to 23 $\mu\text{g/L}$, when the aluminium was given in the form of aluminium citrate (Slanina et al., 1986).

The extent of citrate enhancement varies as a result of experimental conditions. Thus, in one human study, citric acid increased the systemic absorption of aluminium from antacids by about 50 times (Weberg and Berstad, 1986) and in another by 8 times (Walker et al., 1990). Taylor et al. (1992) gave a group of controls and AD patients an aluminium citrate drink and found increased aluminium absorption in both young and old AD patients and also in the older group of controls over that of the young control group.

Several reasons and hypotheses have been offered to explain why aluminium citrate is more easily absorbed than most other aluminium species. (1) It has a smaller size and is more hydrophilic than other forms of aluminium. (2) It may form an uncharged species better able to cross the gastrointestinal mucosum (Slanina et al., 1986). (3) Because citrate chelates endogenous mucosal calcium, it may make the paracellular pathway (between cells rather than through cells) more accessible by opening the tight junctions between the mucosal cells of the bowel (Froment et al., 1989). (4) Citrate may help to hold aluminium in solution at the neutral pH of the bowel. However, Powell and Thompson (1993) point out that aluminium citrate forms only below pH 4, predominantly at pH 2.5.

1.5.2 Tea

Tea has been the subject of several aluminium bioavailability studies because of its high aluminium content. Black tea is from the tea bush, *Camellia sinensis*. It grows on acid soil and accumulates great quantities of aluminium in its leaves, from 8,700-23,000 ppm (Coriat and Gillard, 1986). [Cranberry is the only other plant food known to tolerate such high aluminium levels].

Black tea leaf infusions contain 40-100 ppm aluminium (Baxter et al., 1989). Flaten and Odegard (1988) suggested that most of the extractable aluminium in brewed teas may be strongly bound to organic species (e.g. theaflavins, thearubigins and other polyphenolic 'fermentation' products) and that these high molecular weight complexes are not readily absorbed from the gastrointestinal tract.

Powell et al. (1993) used an *in vitro* model to investigate physiological digestion of tea with human gastric juice and to follow the fate of polyphenolics from tea in the human gastrointestinal tract by looking for low molecular weight breakdown products. The aluminium concentration in their tea infusion was 109 μM of which 76.4 μM was bound to a fraction of less than 3,000 Da at low pH. When the pH was raised to 6.5, the aluminium distribution was associated with higher molecular weight species but the amount of total aluminium in solution was unaltered. Thus, it was considered unlikely that the higher molecular weight form of aluminium resulted from precipitation (Powell et al., 1993).

The tea was given to an ileostomy patient, but H-NMR spectroscopy revealed no significant breakdown of tea-derived polyphenols to low molecular weight phenols during transit of brewed tea through the human small intestine. Although no increase was found in total aluminium excretion following tea versus water drinking the total volume of urine was greater after tea drinking. Drewitt et al. (1993) also reported finding no evidence of increased aluminium absorption from drinking tea with or without added lemon.

Fairweather-Tait et al. (1991) gave young rats, on low-iron diets for 28 days or a diet containing a normal iron level, a tea infusion and measured their blood and liver aluminium levels. The tea further depleted iron but no increase was seen in blood or liver aluminium levels. The authors concluded that aluminium in tea was very poorly absorbed but that tea tannins, either in the form of an infusion or as tea leaves, had an adverse effect on iron status.

In contrast to these results, Koch et al. (1988) fed human volunteers 1.2 litres of tea, coffee or tap water on separate days along with standardised meals. All urine passed in the 12-hour period was collected separately and assayed for aluminium using GFAAS. The amount of aluminium increased in every case on the day tea was taken. They concluded that some aluminium present in the tea was absorbed and that tea consumption should be included in any assessment of the total dietary intake of aluminium in humans.

1.5.3 Minerals

When sodium was present in the intestine with aluminium, only 2 $\mu\text{mol Al/L}$ was absorbed into the bloodstream whereas 100 $\mu\text{mol Al/L}$ was absorbed in the absence of sodium (Van der Voet and De Wolff, 1987). Both calcium and ferrous iron compete with aluminium and inhibit its absorption while calcium and iron deficiency states promote its absorption (Cannata et al., 1991; Provan and Yokel, 1989). Other elements believed to limit the systemic absorption of aluminium are dietary inorganic phosphate (Ondreicka et al., 1966), silicic acid (Edwardson et al., 1993b), fluoride (Ondreicka et al., 1986), and phytic acid, the hexaphosphate of myo-inositol which is particularly present in cereals (Erdman, 1981).

1.6 Aluminium Retention in Tissues

Nutritional conditions not only affect aluminium absorption but also its deposition in tissues. Yasui et al. (1991) showed that aluminium content in bones was higher in rats fed calcium-deficient or calcium- and magnesium-deficient diets with or without added aluminium. Using a very low dose (3.8 ng) of the radioactive tracer aluminium-26, Jouhannau et al. (1993) found as much aluminium deposited in bone from a single oral dose as the amount excreted.

According to Ondreicka et al., (1966), the toxic effects of aluminium salts on bone are primarily due to disturbance in phosphate metabolism, creating a negative phosphorus balance in the rat. In rats fed 5% aluminium sulphate, phosphorus was diverted to the faeces, resulting in a decreased plasma level of inorganic phosphorus with a concomitant fall in femoral bone phosphorus levels. When administered as aluminium phosphate, which is poorly absorbed, the effect was less prominent.

Rats given 281 mg/kg aluminium hydroxide by gastric feeding five times a week for five weeks also received either ascorbic acid, citric acid, gluconic acid, lactic acid, malic acid, oxalic acid, or tartaric acid in the drinking water. Controls were given aluminium hydroxide only. All these food acids significantly increased the aluminium concentrations in most of the tissues, with ascorbic acid and citric acid producing the greatest effect (Domingo et al., 1991b).

Slanina et al. (1985) fed rats a black current soup which was heated and stored for 19 days in an aluminium saucepan. This treatment contaminated the soup, causing it to contain 17 mg aluminium per litre. Rats fed the black currant soup over an 11-week period showed no significant differences in tissue aluminium content from rats fed the same soup cooked and stored in a stainless steel saucepan when measured by GFAAS.

In another study, rats were given 10% v/v ethanol for 6 weeks in combination with 25 mg/kg aluminium nitrate in their drinking water. This produced liver and brain enzyme changes, and depletion in the neurotransmitters 5-hydroxytryptamine and dopamine when compared to rats given aluminium alone. Blood, liver, kidney and brain aluminium levels were also higher (Flora et al., 1991).

2. EXPERIMENTAL PROCEDURES

The purpose of the present study was to determine whether ordinary foods or beverages co-ingested with drinking water aluminium affect its absorption and bioavailability. The intact rat gastrointestinal tract was essentially used as a biochemical reaction chamber. The study was carried out in three stages. The first stage was to determine a suitable oral dose of aluminium in water which could be measured by graphite furnace atomic absorption spectroscopy. In the second stage, beverages were added and, in the third stage of this study, solid foods were also tested for their possible influence on aluminium absorption from water.

2.1 Quality Control

Due to the widespread prevalence of aluminium from dust and other sources, it is very important in experiments of this type to be aware of possible sources of aluminium contamination and to take measures to avoid it. To assess quality control, aluminium measurements were made on all solid materials and non-blood fluids pertinent to the blood sampling, collection, storage and transport aspects of the experiment, prior to beginning the

actual blood collections. To inspect for possible aluminium contamination of plastic containers used throughout the experiment, purified water was left in contact with the internal sides of the containers for 5 minute periods prior to measurement. Repeat measurements on the various materials were also made during the course of this reporting period.

2.2 Experimental and Control Animals

The experimental protocol for this study was reviewed and approved by the Animal Care and Experiment Ethics Committee of the CSIRO Division at Prospect, NSW. Approximately one hundred and fifty adult male Wistar rats, average weight 505 grams, were used for this experiment. They were maintained in the Rat Colony of the Australian Institute for Biomedical Research Ltd. where they were fed *ad libitum* with a standard (non-breeding) maintenance diet containing 13% protein (Gordon Stockfeeds, Yanderra, NSW) and tap water.

The main control group consisted of rats given a single drink, by gastric gavage, of 2 mls of water to which alum had been added. The stock solution was made up by weighing out 8.91 g aluminium sulphate (m.w. 594.29) into 100 mls purified water. This makes a 0.15M stock solution of alum. As there are two aluminium atoms in this molecule, they comprise 54/594.29 or 0.091 of the molecular weight. Hence, $0.091 \times 89.14 \text{ g/L} = 8 \text{ mg}$ elemental aluminium per ml. The osmolarity of the stock solution was 393 mOsM. It was tested each time it was used to ensure that the alum solution was consistent from one experiment to the next. One ml of the 8 mg/ml stock solution was added to one ml of purified water so that the 2 mls of 4 mg/ml water contained a total dose of 8 mg elemental aluminium for the "alum only" controls.

Additional rats served as "food and beverage controls", receiving a dose of 1 ml food or beverage plus 1 ml water without added alum (Table I). The experimental animals received a single dose of a 1:1 mixture of water containing one ml of the 8 mg aluminium dose together with one ml of either an homogenised food or a beverage.

I
PREPARATION OF TEST AND CONTROL DIETS

	Food Homogenate	Beverage	Water	Water & 8 mg Al
Foods (exp)	1 ml			1 ml
Beverages (exp)		1 ml		1 ml
Foods (cont)		1 ml	1 ml	
Beverages (cont)	1 ml		1 ml	
Al only (cont)			1 ml	1 ml

An additional set of controls concerned the test animals themselves. Their blood aluminium levels were measured prior to administration of the alum dose. For each dietary regimen, relative aluminium bioavailability was determined by collecting a timed sequence of blood and urine samples after dosing and measuring with GFAAS their aluminium levels.

2.3 Test Foods and Beverages

The foods tested for the database included apple (as an example of a fruit) broccoli (a green vegetable), lean beef mince (protein), Vita Wheat biscuits (carbohydrate/cereal product), butter (natural fat, Allowrie) and margarine (synthetic fat, Black and Gold). The beverages tested were freshly squeezed orange juice, Coca Cola, tea (Lipton's Jiggler Tea), beer (Tooheys draught), wine (Mt. Bingar moselle), and coffee (Nescafe). The abbreviations are "Ap", "Bc", "M", "VW", "Bt", "Mr", "OJ", "CC", "T", "Br", "W", and "Cf", respectively.

2.4 Oral Dosing

In order to test the effect of the diets on aluminium in drinking water it was first necessary to clear the normal feed from the rat stomach and duodenum (upper intestine). Early in the study, some rats were fasted for 24 hours, sacrificed, and found to still have substantial amounts of food in their stomach at that time, indicating that digestion of the previously-fed pelleted food was continuing. Hence, a decision was made to extend the fasting period.

Thus, for the beverage experiments and "aluminium only" controls, the rats were deprived of their normal food for 36 hrs during which time they were given purified water containing less than 0.1 μmol aluminium per litre. For the food protocols, the rats were given the food to be tested throughout the 36-hour pre-treatment period. This was done to load the gastrointestinal tract with the single food and its breakdown products.

One group of animals was given both a beverage and a food with their alum dose. Consistent with the other food groups, these animals received the test food during the 36-hour pre-treatment period.

After the 36-hour feeding period of the test food or fasting, a portion of the test food was blended in water and provided as an homogenate either with purified water to which alum was added or purified water by itself (Table I). The purpose of the blending was to simulate food that had been chewed. The food was provided as an homogenate in a 1:1 mixture with water or with the aluminium stock solution. All experimental and control feedings were administered directly into the stomach using a 3.5 inch gastric feeding needle.

Animals were weighed just prior to their first blood sample collection. For each dietary protocol, an initial blood sample was taken and designated "time zero", T_0 . The blood sample was collected from the tail vein. For this, a 2-3 mm piece of tissue was removed from the tip of the tail. The tail was held vertically and "milked" by gently squeezing around its circumference while moving the hand downward toward, but not touching, its tip.

Approximately 20-30 microliters of blood were collected directly into a clean Eppendorf centrifuge tube. This first sample served as a baseline value, or control, for each animal's subsequent samples.

The rat was then anaesthetised with 60 mg/ml pentobarbitone using the initial dosage of 1 ml per kg. Anaesthesia was maintained throughout the remainder of the collection period with supplements (1:10 dilution) at intervals as required. In order to sustain body temperature during this time, the animal was placed on its back on a heating pad and covered with a light thermal material. Its core temperature was measured periodically with a digital thermometer inserted into the rectum.

The skin of the neck was clipped, the animal was tracheotomised through an incision in the skin and neck and a cannula located to permit uninhibited respiration. To maintain fluid

balance, a fine-gauge cannula (I.D. = 0.5 mm, O.D. = 0.8 mm) was put into the jugular vein and sterile isotonic saline plus heparin¹¹ was infused at the rate of 2 ml/hour.

Additional blood samples were collected from the tail vein, beginning 1 hour after the oral dosing. The blood was allowed to clot and the tubes were spun down at high speed (13,000 rpm) for 3 minutes in order to extract more serum from the blood clot. The tubes, which were, at the time of collection, labelled "T₀", "T₁", "T₂", "T₃", "T₄" were rearranged and assigned a code letter "A", "B", "C", "D", or "E" so the GFAAS analyst would be blind as to their identity.

Urine samples were also collected at one hour intervals, beginning one hour after the oral gavage. In order to avoid extraneous aluminium contamination, the bladder was punctured with a fine hypodermic needle and the urine drawn up into a 1-ml syringe. The urine volume was recorded from each sampling. The samples originally labelled UT₁", "UT₂", "UT₃", and "UT₄" were scrambled into a different order and likewise coded "UA", "UB", "UC", and "UD". At the conclusion of the sampling period, the anaesthetised animal was given a lethal intravenous overdose of potassium hydroxide. The carcass was frozen and then incinerated.

The blood and urine samples were sent to Royal North Shore Hospital for analysis of their aluminium content using GFAAS. The method of determination that was used complied with Australian Standard CH6 92-5, Determination of Aluminium in Serum--Graphite Furnace Atomic Absorption Spectrometric Method. All materials and solutions involved in the determination were rigorously screened for contamination by exogenous aluminium.

The GFAAS procedure involved diluting fifty or 100 microliters of the rat serum with 100 or 200 microliters of ASTM Type 1 water containing a trace of surfactant. A ten microliter-drop of the diluted serum was placed on the platform of a graphite furnace workhead, dried and atomised. Replicate atomisations, at least, were carried out on all solutions. Aluminium levels were expressed in μmol aluminium per litre of plasma. The measurement results were returned to the AIBR, decoded, and the data for the experiments testing aluminium only or in combination with a food or beverage were passed on to a statistician to calculate the means, the standard error of the means, and carry out analyses based on Wilcoxon tests and Welch modified t-tests.

The urine aluminium values for T₁ through T₄ were each multiplied by their volumes (in mL), combined, and then multiplied by 0.027 (27 being the atomic weight of aluminium) to calculate the aluminium content in micrograms (ppb) excreted over 4 hours.

¹¹Heparin was prepared at a dilution of 1 ml to 4 mls of saline (500 units/5 ml 0.9% NaCl).

The serum aluminium values of the T₁₋₄ hr samples were also added together. The T₀ (baseline) serum aluminium measurement was then multiplied by 4 and subtracted from the T₁₋₄ hr serum value to obtain the 4 hour serum total. To convert this $\mu\text{mol/L}$ value to $\mu\text{g/L}$, it was also multiplied by 27.

3. RESULTS

The data consisted of assayed levels of aluminium in urine and serum samples collected at four one-hourly spaced intervals, together with an initial serum reading taken prior to the gavage. As will be shown, some nutrients were found to enhance "relative aluminium bioavailability" from drinking water while others tended to inhibit the process.

3.1 Aluminium Content of Foods and Beverages

When the foods and beverages were directly tested for their aluminium content, they were found to have a large range of values (Table II). Tea and some beverages stored in aluminium cans had very high aluminium levels.

II
ALUMINIUM CONTENT OF BEVERAGES AND FOODS
WITHOUT ADDED ALUM

Beverage or Food	Aluminium content
Beer (Toohey's, aluminium can)	446 $\mu\text{g/L}$ (lowest)
	8640 $\mu\text{g/L}$ (highest)
Coca Cola (aluminium can)	3240 $\mu\text{g/L}$
Instant Coffee (Nescafe)	97 $\mu\text{g/L}$
Prepared Orange Juice (Quelch)	149 $\mu\text{g/L}$
Fresh Orange Juice	26 $\mu\text{g/L}$
Tea (Lipton's Jiggler Tea)	4797 $\mu\text{g/L}$
Wine (Mt Bingar Moselle)	5 $\mu\text{g/L}$
Apple	90 $\mu\text{g/L}$
Broccoli	270 $\mu\text{g/L}$
Meat (beef meatballs)	39 $\mu\text{g/L}$
Vita Wheat	140 $\mu\text{g/L}$

3.2 Dose Determination and Osmolarity

For this stage of the experiment, rats were given aluminium doses ranging from 0.1mg/ml to 32 mg/ml (Table III). The lowest dose was undetectable with GFAAS. The 1 mg/ml dose produced a small increase but it was insufficient to distinguish from the variation in serum aluminium levels that follow an oral dose of purified water. The 2, 4, 8 and 16 mg/ml doses produced progressive plasma aluminium increases but the 32 mg/ml dose was less, possibly due to precipitation. The most suitable dose for these experiments was determined to be 1 ml of an 8 mg/ml solution so it could be diluted with one ml of pure water (giving a 200 mOsm solution), beverage or food homogenate for a 4 mg/ml final solution. This formula reliably produced a distinct increase in plasma aluminium, usually appearing as a peak over the 4 hours of measurement. It also allowed a consistent amount of aluminium to be added to the experimental protocols regardless of the diluent.

III
EFFECTS OF INCREASING ALUM DOSE

Dose in mg/ml	(mmol/L)	Serum incr ($\mu\text{mol/L}$)	(mOsm)
1	9	0.42*	113
2	28	0.80	151
4	83	1.79	200
8	150	2.10	393
16	280	2.61	811
32	626	1.87	1690 (est.)

*This level was indistinguishable from serum aluminium variations in a rat receiving purified water without added aluminium.

3.3 Absorption of Aluminium from Beverages or Foods Alone

Foods or beverages were given on their own as controls, i.e. diluted with purified water containing no measurable aluminium. Some of them (namely, Coca Cola, orange juice, tea, apple, and margarine) appeared to induce a small transient rise or lowering of the plasma aluminium content while others showed no effect at all. Of these, the most consistent effect came from margarine feeding which produced an elevation of the plasma aluminium level even when aluminium was not added to the water. This increase is seen on Table IV in the first column, "baseline serum aluminium", in the row designated "Al/Margarine". The mean baseline value for margarine was approximately 30 $\mu\text{g/L}$ whereas the mean baseline aluminium measurements for the other beverages and foods (including butter) were less than half as much. The amount of aluminium actually contained in margarine, and butter, were not measured in the GFAAS to avoid damage to the instrument.

3.4 Absorption of Aluminium from Water Containing Alum

When the rats were given 2 mls of the 4 mg aluminium/ml drinking water, aluminium levels peaked in the serum fractions at approximately 1 hour and in the urine at 2 hours from the time of ingestion. With each subsequent hour, the aluminium values tended to fall. Occasionally, the falloff was non-linear and a secondary peak was recorded during the later measurements.

The summary data for all the test diets are shown on Table IV and the mean values for the alum only controls are listed in row 1. As can be seen, the mean for the baseline was 12.52 $\mu\text{g/L}$. The mean for the peak serum aluminium measurements was 56.63 $\mu\text{g/L}$ (data now included). Subtracting this gives a mean increase of 44.11 $\mu\text{g/L}$ as listed in the column designated $\Delta\text{Serum Al}$. The mean 4-hour serum and urinary aluminium measurements were 115.32 and 2.59 $\mu\text{g/L}$ respectively. The test diets were compared against these "Alum only" results to determine their significance. If they increased or lowered the amount of aluminium absorbed relative to the "Alum only" measurements they were considered to have a positive or a negative effect on aluminium absorption and consequent bioavailability.

3.5 Absorption of Aluminium from Water with Alum plus Beverages or Foods

Absorption data for the beverage experiments are summarised on Table IV, rows 2-8. As can be seen from this table, fresh orange juice gave the largest increase in aluminium absorption

IV
ALUM ONLY VS ALUM WITH BEVERAGE OR FOOD

Treatment	No.	Baseline Serum Al (µg/L)		ΔSerum Al (µg/L) Al		4 Hr Serum (µg/L)		4 hr Urine Al (µg/L)	
		m	sem	m	sem	m	sem	m	sem
Alum only	(8)	12.52	3.47	44.11	8.45	115.32	20.22	2.59	0.52
Al/Beer	(5)	14.20	1.55	58.81	6.21	179.44	18.48	1.95	0.62
Al/CCola	(5)	8.32	1.26	54.65	8.42	176.53	29.32	1.60	0.23
Al/Coffee	(5)	3.73	1.08	109.24	18.11	333.13	57.74	3.43	0.40
Al/OJ	(5)	12.58	2.89	744.07	206.50	2114.86	572.02	27.23	9.00
Al/Tea	(8)	12.42	52.11	39.69	3.42	121.36	13.38	2.23	0.28
Al/Wine	(6)	9.36	1.72	82.80	4.99	276.34	16.64	4.56	0.78
Al/Apple	(5)	9.61	1.52	42.77	8.18	147.31	29.19	2.45	0.60
Al/Broccoli	(5)	4.97	1.74	39.47	9.10	129.01	30.49	1.80	0.22
Al/Butter	(5)	8.59	1.79	35.26	4.55	105.19	18.05	2.42	0.31
Al/Margarine	(5)	29.43	4.63	35.75	6.87	91.48	25.52	3.20	0.30
Al/Meat	(5)	14.58	2.64	27.11	4.38	87.32	17.27	1.17	0.24
Al/VW	(6)	8.24	0.96	28.48	5.84	85.77	11.15	1.49	0.44
Al/OJ&VW	(5)	13.50	3.36	401.76	116.49	1172.61	364.21	28.62	13.54

Δ : increase
m : mean
sem : standard error of the mean

and excretion although the fresh juice naturally contains very little aluminium. Coffee and wine also significantly elevated aluminium absorption. Beer produced a small but statistically insignificant effect.

The food plus aluminium data are also shown on Table IV (rows 10-15). Compared to the alum-only controls, the rats fed meat or Vita Wheat biscuits with the alum-water had somewhat lower serum aluminium measurements than alum exposure alone but due to the small difference and size of the groups these results were statistically insignificant.

When the beverage producing the greatest positive effect (orange juice) was given simultaneously with the food producing the largest negative effect (Vita Wheat biscuit), there was a lowering of the mean peak increase in serum aluminium level but no change in the urinary excretion of aluminium. The positive enhancement by orange juice was still prominent.

3.6 Variation in Individual Levels of Aluminium Absorption

A proportion of the rats had unusually high serum levels for any given treatment. In some individuals it was noted that this coincided with a lower renal excretion of the aluminium. An example of the variability between individuals and also between fasting versus fed individuals is shown for animals which received alum along with orange juice (Table V). Of the fasted animals, 6/18 or one third showed substantially higher increases in aluminium serum levels after dosing than the mean value. Another 7/18 had lower aluminium increases. In the fed group, 3/11 animals had higher aluminium readings than the mean increase and 2/11 were relatively lower.

Moreover, fasted rats fed alum plus orange juice had 5.5 times higher aluminium levels in their serum samples than the rats which had been fed prior to the gavage.

3.7 Data Analysis

High levels of aluminium absorption, and hence bioavailability, were found for diets containing orange juice and, despite there being only five-six animals in each group, for coffee and wine. Wilcoxon tests and Welch t-tests were evaluated for several groups (see

V
 VARIATION IN SERUM ALUMINIUM LEVELS IN
 FASTED OR FED RATS GIVEN OJ & ALUM

Fasted versus Fed	ID Number	Baseline Serum Al $\mu\text{g/L}$	Peak Serum Al $\mu\text{g/L}$	Δ Serum Al $\mu\text{g/L}$	
Fasted Animals	4	11.88	159.30	147.42	
	44	13.77	201.15	187.38	
	45	12.42	207.36	194.94	
	24	5.94	227.34	221.40	
	42	16.47	299.97	283.50	
	25	7.83	295.9	288.07	
	41	19.71	337.77	318.06	
	40	15.12	352.6	337.48	
	49	9.99	410.94	400.95	
	50	45.09	563.76	518.67	
	51	11.61	568.89	557.28	
	43	13.50	635.31	621.81	
	17	22.18	966.6	944.42	
	47	12.42	1007.10	994.68	
	15	11.61	1019.25	1007.64	
	48	11.88	1035.45	1023.57	
	46	21.87	1095.66	1073.79	
	16	15.39	1272.24	1256.64	
			278.68 m = 15.48 $\mu\text{g/L}$	10,656.59 m = 592.03 $\mu\text{g/L}$	10,377.91 m = 576.55 $\mu\text{g/L}$
	Fed Animals	59	3.51	35.92	32.40
58		1.62	42.12	40.50	
52		4.86	96.93	92.07	
57		8.91	109.62	100.71	
6		9.99	111.24	101.25	
60		5.40	108.54	103.14	
7		16.74	126.90	110.16	
31		29.70	140.40	110.70	
53		4.86	116.37	111.51	
32		19.71	135.00	115.29	
54		4.05	136.35	132.30	
56		5.40	159.57	154.17	
55		4.86	173.88	169.02	
			4.43 $\mu\text{g/L}$ m = 9.20 $\mu\text{g/L}$	1492.83 $\mu\text{g/L}$ m = 114.83 $\mu\text{g/L}$	1373.22 $\mu\text{g/L}$ m = 105.63 $\mu\text{g/L}$

Tables IV and VI). There was a high correlation between the total 4 hour urine output and peak increase in serum aluminium level ($r = 0.82$ and Fig. 1).

VI
 SIGNIFICANCE OF SELECTED DIETARY TREATMENT
 DIFFERENCES FROM ALUM TREATMENT ONLY

Treatment	P ₁	P ₂	Significance
Orange Juice :	0.002	0.0003	+
Coffee :	0.011	0.009	+
Wine :	0.013	0.008	+
Meat :	0.241	0.163	-
Vita Wheat :	0.181	0.190	-

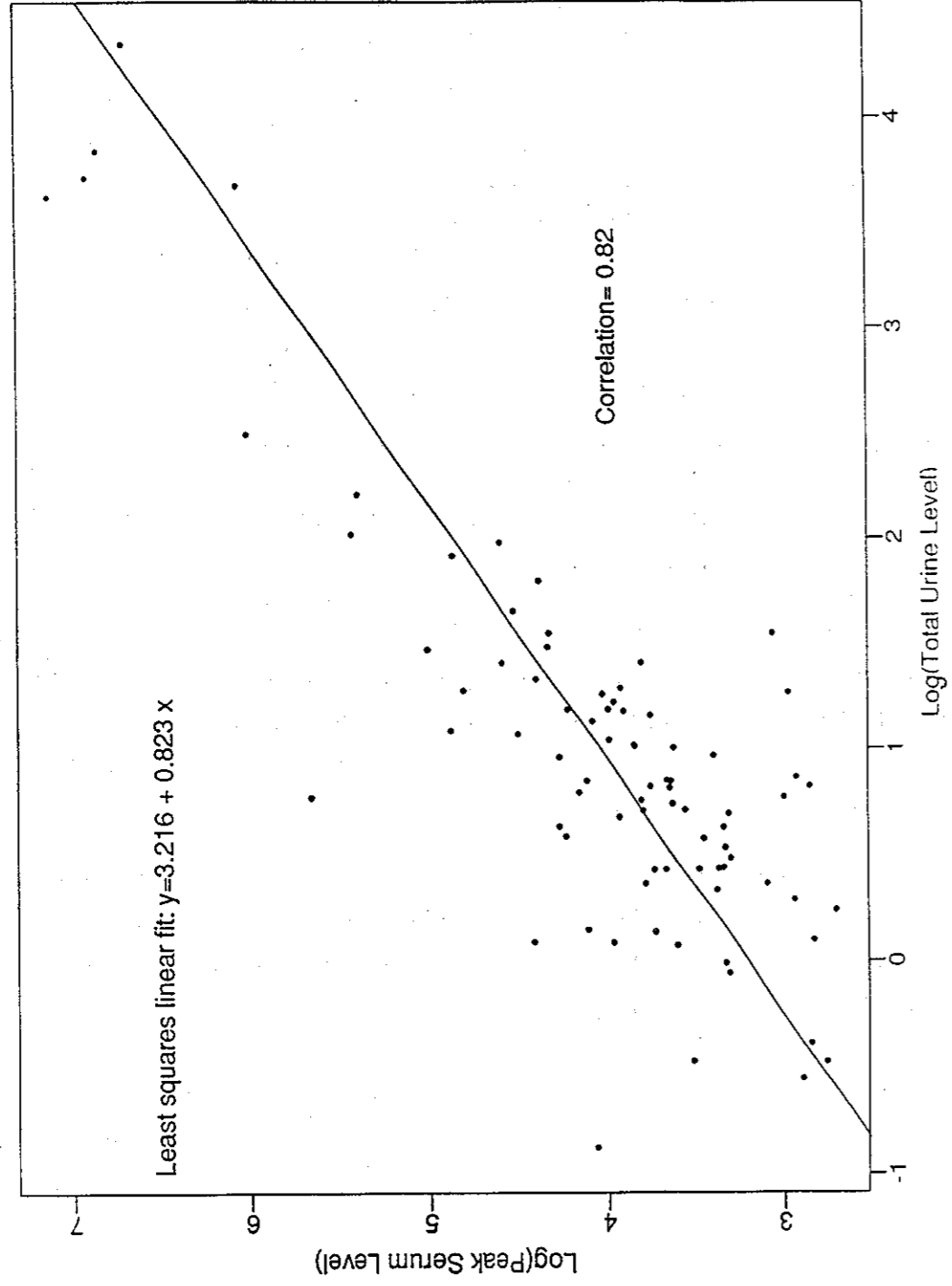
P₁: Probability from Wilcoxon rank sum statistic

P₂: Probability from a Welch modified t-test on the logarithms of the peak aluminium levels

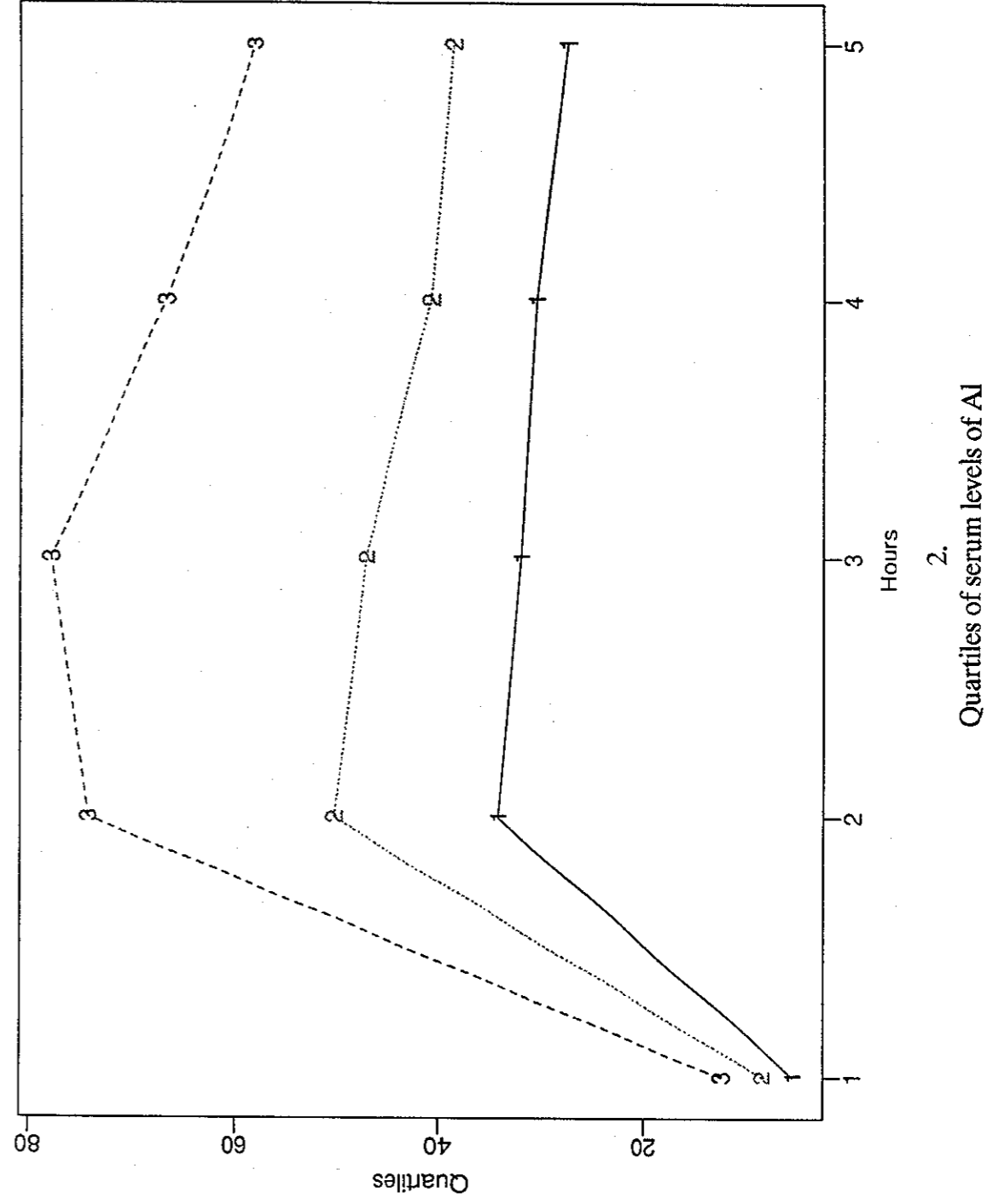
Figures 2 and 3 show responses in time by plotting the quartiles of the distribution of serum aluminium and total urine levels. The asymmetrical placement of the upper quartile with respect to the median compared to the lower shows the skewness in the combined data. Typical responses in time (measured over 4 hours), as judged by the quartiles at each hour, show that serum level peaked for most rats at 1 hour and urine at 2 hours post-ingestion.

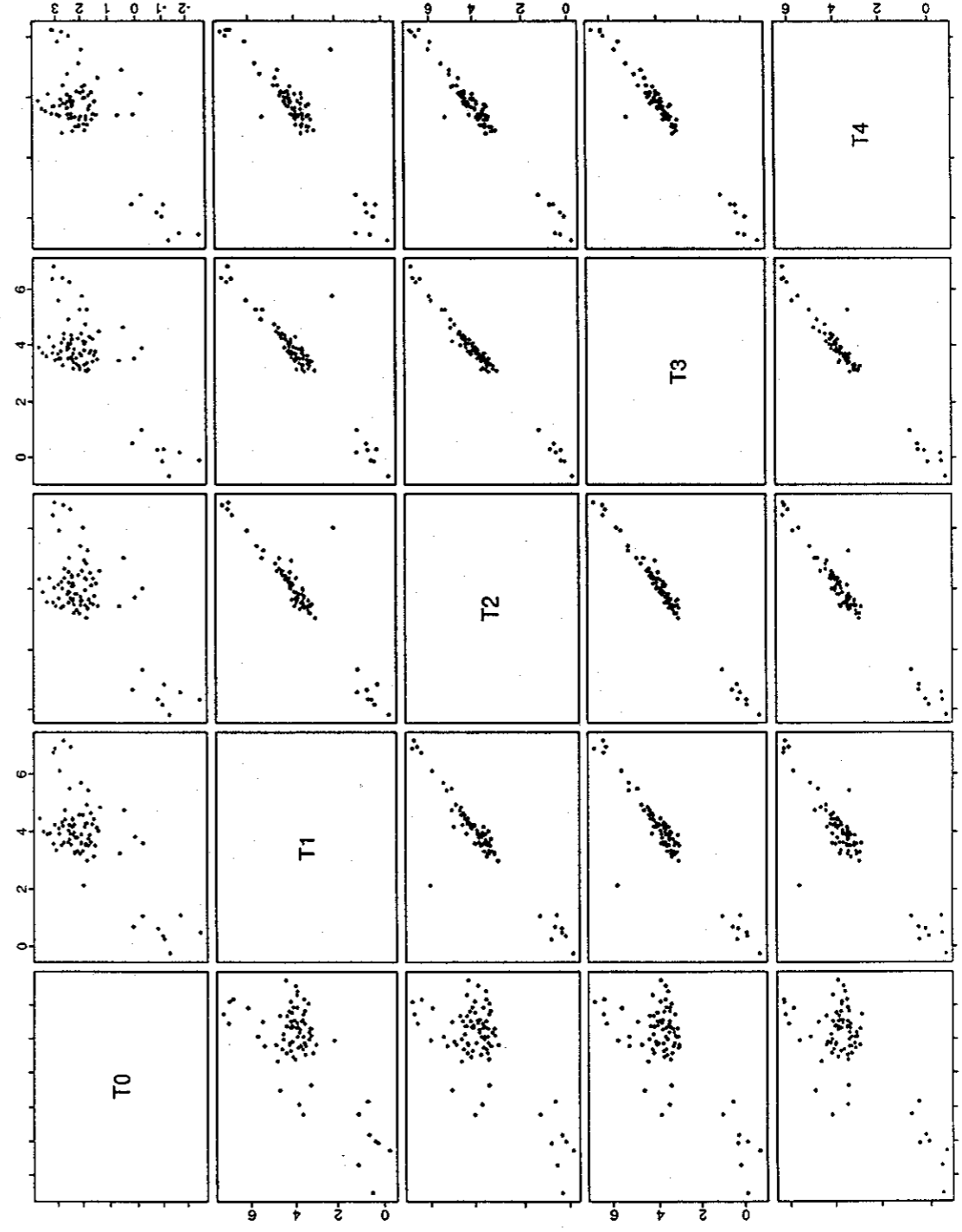
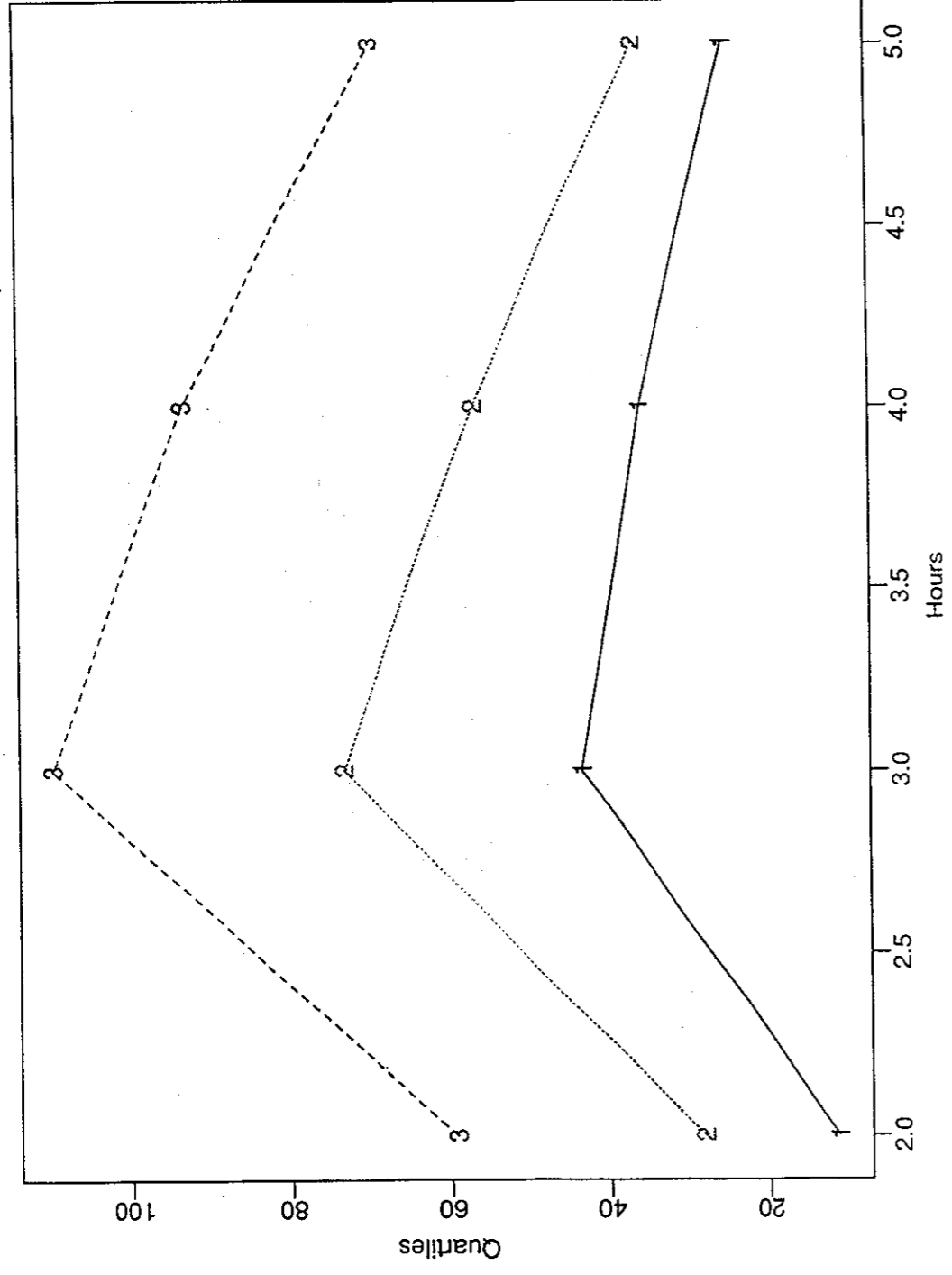
The relationships between the log(serum levels) at each time are shown in Fig. 4. There is a relationship between initial level (T₀) and subsequent levels (T₁ to T₄). Between T₁ to T₄ there is strong association. There are a couple of atypical points evident in the relationships between the hours (see Fig. 4).

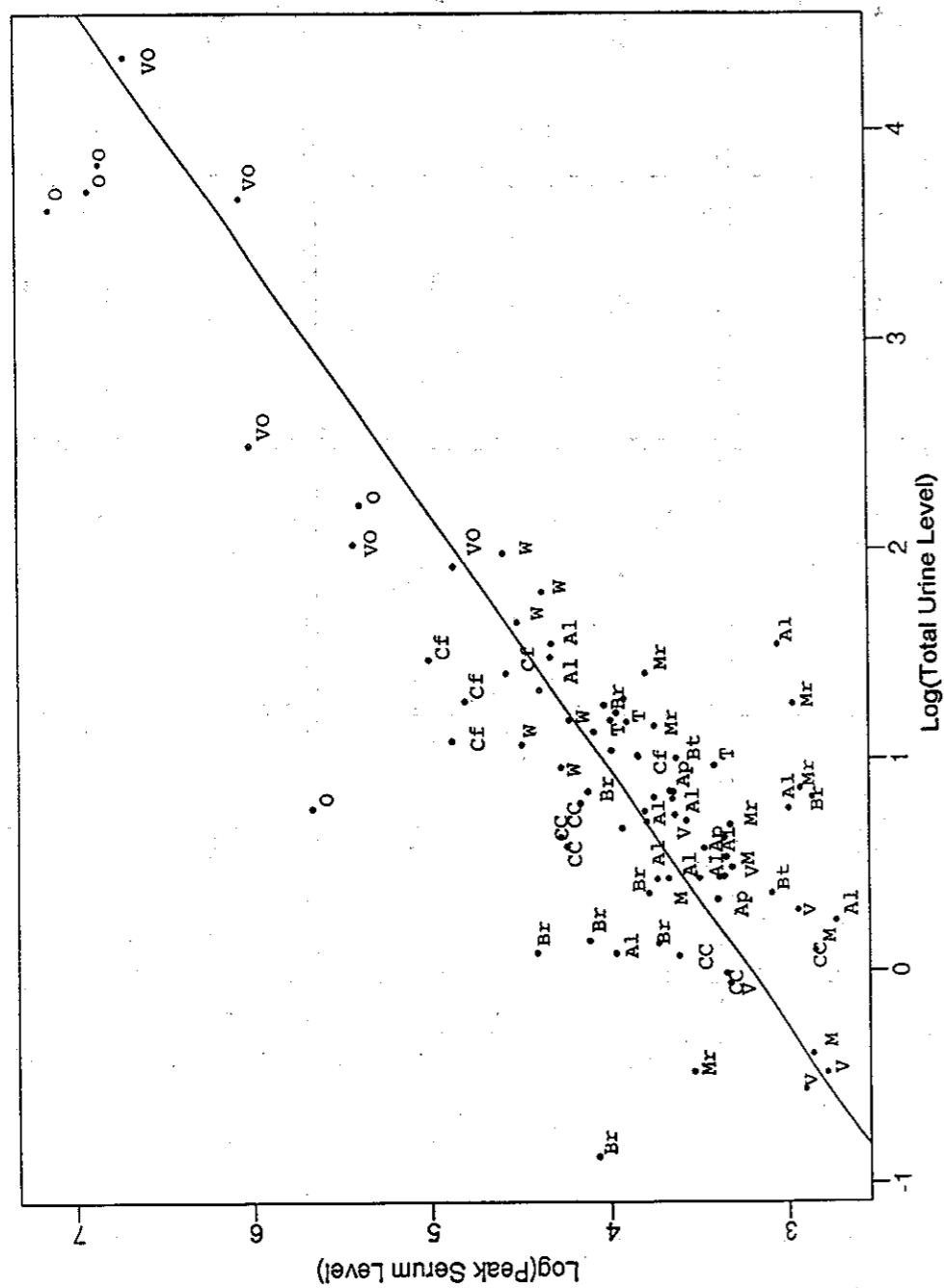
Fig. 5 shows the relationship between the log(increase (D) in peak serum Al) and log(total urine Al) with the high and low extremes indicated by their diet code, with clear clumping of the "O" (Al with OJ), "VO" (Al with Vita Wheat & OJ), "Cf" (Al with coffee) and "W" (Al with wine) diets at the high levels. The "Al" diets (Al with water) are amongst the low values but have a fair spread of values while the "M" (Al with meat) and "V" (Al plus Vita Wheat) are at the lowest end of the scale.



1.
Correlation between peak serum Al and total urine Al on the log scale







5. Effects of the various test diets on Al absorption

4. DISCUSSION

The present study required a large number of samples to be measured for each test diet and so only a limited selection of the many foods that humans consume were tested. Nevertheless, the results allow insight into some of the conditions which may affect aluminium bioavailability from drinking water. The present "relative aluminium bioavailability" database can serve as a nucleus which hopefully will continue to expand.

This data, together with research findings from previous investigations by ourselves and others, fundamentally contradict two generally believed assumptions about aluminium metabolism:

1. The first incorrect assumption is that *The amount of aluminium absorbed from an ingested substance is directly proportional to the amount of aluminium it contains.* Because alum-treated water contains less aluminium than that naturally contained in most foods, the contribution of water to the body's aluminium burden has often been dismissed, notably in official water reports (e.g. WHO "International Standards for Drinking Water," Geneva, 1984).

Examples were given by us and other research findings were described in the Introduction section of tea infusions which contained high levels of aluminium but had little effect on aluminium absorption. Coca Cola also had a high aluminium level when tested, possibly due to defects in the resin lining of its aluminium container. Despite that, and its low pH value, conditions which would be expected to increase the solubility of aluminium salts and to promote aluminium absorption, Coca Cola produced no measurable effect.

In contrast to those results, orange juice, which has a relatively low aluminium content, given together with alum in water, caused plasma aluminium levels to rise significantly higher than when the orange juice or the alum drink was given on its own. The same results occurred for coffee and wine. Thus, dietary choice regulates, to a surprising extent, the amount of bioavailable aluminium that one absorbs.

These results show that there is no fixed relationship between the aluminium content of a nutrient and the amount that will be taken up into the body following its ingestion. This

means that the simple formula (2 litres 0.2 mg/L x 1%) used to estimate daily aluminium bioavailability from drinking water is virtually meaningless. The actual data indicate that total aluminium absorption from drinking water is considerably more complex than assumed.

More important than the aluminium content are the conditions under which the alum-treated water is drunk. Considerably more aluminium was absorbed when the beverage was taken without food present in the stomach to buffer the pH and compete for the aluminium ions. Breakfast is a prime time for aluminium absorption from the morning coffee or orange juice. In humans, stomach contents usually clear much more quickly than in rats--in the order of one-few hours--depending upon what has been consumed.

One possibility that should be discussed here is whether, in the beverage experiments, the 36-hour fasting period may have thrown the body's physiological mechanisms into a "starvation state," causing an artifactual increase in the amount of absorbed aluminium. Our data is unresponsive of this hypothesis for four reasons:

- 1) the animals were not deprived of food for the entire 36-hour period since a substantial amount of food was still present in the upper part of the digestive tract of animals fasted for 24-hour periods;
- 2) all animals given beverages with the alum were fasted and the enhanced effect occurred only in the presence of some beverages but not others;
- 3) when rats were fed with Vita Wheat biscuits during the 36 hour period prior to receiving the biscuits with orange juice plus alum, aluminium absorption increase was still very pronounced.
- 4) the high correlation ($r = .82$) between the urine and serum aluminium measurements for all treatments indicate that the kidneys were not in a urine dilution mode.

Some explanations come to mind for the effects observed by the various test diets. Citric acid is the main acid in oranges as well as in lemons and limes. The citric acid content of orange juice is approximately 7g/L (Igor, 1989). It has been suggested (Domingo et al., 1991b) that

ascorbic acid and citric acid both inhibit the precipitation of aluminium within the intestine, thereby increasing its solubility.

Wine may alter the permeability of the GIT lining by interacting with its mucosal cells.

Coffee and tea both contain caffeine and tannic acid but they produced different effects. Coffee also includes 10-15% coffee oil, 8% sucrose and other sugars, 11% proteins, 5% ash, chlorogenic acid, cellulose, hemicelluloses, trigonelline, and volatile oils (Budaveri, 1989) but it is not clear which of these interact with aluminium to increase its absorption.

Conversely, some solid foods apparently bind with aluminium, making it is less readily absorbed and/or buffer the stomach acid to inhibit aluminium absorption in that way (Powell and Thompson, 1993). Minced beef seemed to lower aluminium absorption but we have no hypotheses on the reason for this possible effect. The Vita Wheat biscuits also tended to inhibit aluminium uptake. There are at least two possible explanations for this observation. One may due to the high content in wheat of phytate. This ingredient has been predicted to chelate aluminium and inhibit its absorption (Powell and Thompson, 1993) but this was apparently the first time the hypothesis has been tested. Also, silica is found at relatively high levels in cereal grains and silicic acid is known to inhibit the absorption of aluminium from water in the digestive tract (Edwardson et al., 1993).

2. The second incorrect assumption is that *Aluminium addition to drinking water (and also to foods and medications) is safe*. The arguments on which this is based is that aluminium is poorly absorbed and that the small amounts which could get into the body would be easily and totally removed by the kidneys.¹⁹⁻²¹ Instrumentation routinely used to measure aluminium in blood plasma is too insensitive to follow the physiological quantities of aluminium continuously absorbed. More recently, sophisticated instrumentation has been used to show that some aluminium can directly enter the brain from a single drink of water even at the trace levels normally contained in tap water (Walton et al., 1994). These are cumulative over a life span. If the level of drinking water aluminium taken up by the rat brain can be extrapolated to humans it can largely account for the higher aluminium level contained in the aged human brain (McDermott et al., 1979). It is, therefore, necessary to develop an improved understanding of aluminium metabolism in the healthy body, particularly in aged subjects who are at increased risk for dementia.

Alum-treatment of drinking water began in the late nineteenth century and the process was widely adopted in the first few decades of the twentieth century. Over the past 15-20 years, many reports have appeared in the scientific literature describing aluminium toxicity in the brain and other tissues of kidney failure patients. Research attention is now focusing on the absorption of aluminium in healthy individuals. To date, the water industry appears to have ignored the substantial scientific literature on this subject and the potential risk for the general population.

The present study identified a significant population of the animals (approximately 30%) which absorbed considerably more aluminium than the others. Similarly, in the radioactive aluminium experiment cited above, a sub-population of rats had higher aluminium levels in their brain following the single drink of water (Walton et al., 1994).

We also reviewed human aluminium data in the literature (Taylor et al., 1992; Harrington et al., 1994) where individual measurements were given and noted that the same trends were present. One study gave values for the gastrointestinal absorption of aluminium citrate, and the other quantified aluminium content in the brain of demented and non-demented dialysis patients. The subpopulations with increased aluminium levels and the amount of variability (co-efficient of variation) in our studies was consistent with that seen in the human data. These differences probably reflect genetic variation between individuals. Calcium- and/or magnesium-deficiencies, if present, could also exacerbate aluminium absorption and bioavailability and so could dietary habits. The elevated plasma aluminium levels would place the high aluminium responders at greater risk to brain cell loss and other aluminium-induced pathologies.

5. CONCLUSIONS

Aluminium is known to be toxic to the tissues of plants, animals and humans. When present in sufficient concentrations (which are still small) it damages brain cells and interferes with bone growth. Despite this, it has been widely added to water and to food. Alum addition to water in the clarification process increases the ratio of soluble or bioavailable aluminium to insoluble particulate aluminium species (Tran et al., 1993). Even at the trace levels of aluminium that might be found in tap water, some aluminium can pass directly into the brain. The long-term health consequences of this alum-based water treatment are currently unknown but must be regarded as potentially dangerous.

From this research the following conclusions can be reached:--

1. Previous estimates of aluminium bioavailability from drinking water have been considerably oversimplified and probably underestimated. They did not recognise the diverse circumstances under which water is consumed and particularly the effects that other nutrients have in altering aluminium absorption.
2. When ingested with water some foods or beverages promote aluminium absorption from the water and others inhibit its absorption. This bears no obvious relationship to the quantity of aluminium that is present in the food or beverage by themselves. This further emphasises that it is not the total amount of aluminium in the diet that is important but rather the chemical interaction between its various constituents of which drinking water, containing bioavailable aluminium, plays a significant part.
3. Some rats for genetic reasons absorb two to four times more aluminium than others and up to 10 times more by the time it is taken into the brain. Human aluminium data appears to show the same trend. This implies that the same amount of ingested aluminium could give a higher risk of toxicity to a genetically-predisposed proportion of the population.

6. RECOMMENDATIONS

This project and other research around the world suggest that the use of alum in water treatment could have implications for water authorities and that further research is required.

Further research to ascertain whether there is a health related risk to customers from alum in water treatment should be supported by the water industry as a matter of urgency. Examples of such projects might be:

1. Identification of percentages and kinds of soluble aluminium species (Al fluoride, Al phosphate, Al hydroxide, Al silicate, and Al sulphate) in different tap water. Their relative absorption levels in experimental animals can then be assessed.
2. At the sub-cellular (electron microscopic) level, an evaluation of the threshold level(s) of aluminium that is required to start to produce toxic changes in brain cells. If this concentration is found to be in the order of magnitude of that which is known to exist in the ageing human brain, then it would be prudent for the water industry to be very circumspect about its use of alum.
3. Enlarging the relative aluminium bioavailability database so as to improve the understanding of aluminium absorption from the normal diet.
4. Giving, over their lifespan, purified water without aluminium to one group of rats and purified water to which tap level amounts of aluminium have been added to another group of rats, and comparing their memory performance well into old age.

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