ALUMINIUM BONE DISEASE IN PATIENTS RECEIVING PLASMA EXCHANGE WITH ALBUMIN CONTAMINATED WITH ALUMINIUM AND TRACE METALS

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GENERAL AIMS

To study the effect of albumin contaminated with aluminium on the development of bone disease in patients with impaired renal function and to evaluate the interaction of citrate with aluminium and other metal ions in albumin solutions.

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DECLARATION

I hereby declare that this thesis has been composed entirely by myself and that the studies contained herein have been conducted solely by myself. Where collaboration with others was involved this is defined and acknowledged in the relevant chapters.

SUMMARY

Human albumin, and other widely used blood products which are administered by injection, were analysed to determine the extent of aluminium and other trace metal contamination. Increases in metal ion concentrations from ten fold to several thousand fold the normal reference values were detected.

Detailed metabolic balance studies were undertaken on eight patients who were receiving repeated plasma exchange as treatment for a variety of illnesses using commercial albumin solutions known to be contaminated by trace metals. The quantitative retention and excretion of aluminium were measured in these patients and related to the degree of renal impairment. The excretion of this metal was proportional to creatinine clearance, with significant retention of an aluminium load in patients with poor renal function. The incidence of aluminium related bone disease was assessed from the clinical history, bone biopsy histomorphometry and administration of desferrioxamine as a mobilization test. Three of the four patients with poor renal function had bone disease.

The citrate anion which is used as an anticoagulant during the collection of whole blood donations was found to be present at high concentration in the final albumin product. Metal citrate complexes are formed during the

blood fractionation procedure, particularly during the depth filtration stage. These low molecular weight metal species are now being removed by the manufacturer after introduction of an additional ultrafiltration step which produces a 'clean' product. The efficient renal excretion of aluminium and other metal citrate complexes has limited clinical toxicity following the injection of these contaminated substances, provided the patients have reasonably good renal function. A balance study on one patient who was treated with the newly produced clean product has confirmed this view.

CHAPTER 1.

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CHAPTER 1 INTRODUCTION

1.1 GENERAL INTRODUCTION

Aluminium has been shown to be the toxic agent in the pathogenesis of dialysis encephalopathy, Vitamin D resistant osteomalacia and a hypochromic microcytic anaemia in patients with renal impairment (1). In addition there is epidemiological and scientific evidence linking aluminium as a possible toxin in Alzheimer's disease, (2)(3) although its role as a primary cause in this widespread disorder is much disputed (4)(5)(6)(7).

Aluminium is the most abundant metal and the third most common element in the environment. Nevertheless, toxicity from this ubiquitous metal is relatively uncommon. An explanation lies in the very effective barriers which have been developed by man during the process of evolution to exclude this metal from the internal environment. The gastrointestinal tract, lungs and skin provide an almost impenetrable barrier to aluminium and if any of the metal does pass this barrier then it is dealt with by a most efficient renal excretion system.

Over the last fifty years industrial and domestic use of aluminium has increased considerably and these barrier mechanisms have been severely tested. In more recent years man has found even better ways of by-passing these natural defence mechanisms and has introduced aluminium into the human body by the addition of aluminium

salts to pharmaceuticals.

The most clinically important source is aluminium-contaminated water used in the preparation of renal dialysate. Large amounts of oral aluminium hydroxide are also administered as antacids and used as phosphate-binders in patients with impaired renal function. It took some time before the medical and scientific world recognised these potential sources of aluminium toxicity and took effective action to minimise poisoning in the 'at risk' population.

Another important source of aluminium intoxication has received little attention. Albumin solutions are widely used as plasma expanders and it is estimated that over ten million grammes of this protein solution is infused into patients in one year.

Albumin was first shown to be contaminated with aluminium in 1978 (8) and since then manufacturers have claimed that the aluminium concentrations present in these solutions are too low to pose a threat. They have argued that the volumes administered are so small that the risk is negligible compared to environmental sources of aluminium and aluminium-containing drugs such as phosphate-binders. The manufacturers of albumin solutions have put the onus on clinicians to show evidence of aluminium toxicity in patients receiving these solutions. It is true that many

patients receive small volumes of albumin and therefore the risk of toxicity may be low but there are many patients, and particularly those with impaired renal function, who may receive large quantities of albumin over many years. One such group comprises patients receiving chronic intermittent plasma exchange.

Albumin solutions and other blood products marketed by all the major manufacturers in the United Kingdom were studied for evidence of contamination with aluminium and other trace metals. Detailed metabolic balance studies and bone biopsies were performed on patients receiving albumin solutions to determine whether or not they developed aluminium bone disease.

The results of these investigations were made available to the manufacturers of the products examined. One of these manufacturers, the Protein Fractionation Centre, Scottish National Blood Transfusion Service responded to the report and collaborative studies were set up to determine the sources of metal contamination. The possible role of citrate-metal complex formation in enhancing renal excretion of these metals was evaluated.

1.2 HISTORICAL BACKGROUND

Aluminium derives its name from alum which was used medicinally as an astringent in ancient Greece and Rome (Latin alumen: bitterness). The impure metal was first isolated by the Danish scientist M C Oersted in 1827 and it became commercially available in 1854. So precious was the metal at this time that it was exhibited next to the crown jewels at the Paris Exposition of 1855 and the Emperor Louis Napoleon III used aluminium cutlery on State occasions.

Charles Dickens predicted that this newly available metal would soon displace iron and copper in kitchen utensils and that respectable babies would be born with aluminium spoons in their mouths. A dramatic thousand fold drop in the price of aluminium occurred before the end of the century. This was due to the advent of cheap electricity and advances in the purification techniques for aluminium. World production rose quickly and aluminium became available on a massive scale.

Aluminium is the most abundant metal in the earth's crust (8.3% by weight) and industrial use of this metal exceeds ten million tons per year. Daily exposure to aluminium, either from environmental or industrial sources, is virtually unavoidable. Whether such exposure to aluminium is a threat, or is innocuous, or is even necessary and

beneficial to human health are questions which have as yet not been completely resolved.

Throughout the century since the metal has appeared in commercial quantities and as its use in food packaging, cooking utensils, beverage cans and piping has expanded there has been concern about its possible toxicity.

In 1913 the corrosion of cooking pots as a potential source of aluminium was debated in several leading articles in the Lancet(9)(10). Their conclusions were reassuring as were those of many other investigators in the first half of this century.

One of the first reports of poisoning which was attributed to aluminium was in 1921 (11). The patient, a metal worker, had loss of memory, tremor, jerking movements and incoordination. Five years later attention was drawn to the potential health hazards of the aluminium present in city drinking water and various medicines(12). This warning was largely ignored and in the 1940's inhalation of aluminium powder became popular as a method of treating silicosis. Patients were exposed to regular inhalations of fine particles of aluminium for periods of many years without any reported adverse tissue reactions. The procedure was apparently abandoned because of doubts about its efficacy and its unpleasant nature rather than for fears of toxicity. It is said that 30,000 workers

survived the treatment.

In 1957, a team of environmentalists from Cincinnati studied over 1500 books, articles and other manuscripts and wrote an exhaustive review of the 500 most informative(13). In 1974, another team from the same department extended the review to a further 800 papers published in the intervening 17 years(14). At the end of each review they concluded that there was little need for public alarm and that no major health hazard had emerged despite very extensive use of aluminium.

They did however acknowledge the deficiencies of many of the methods used to measure aluminium in the studies they reviewed and much of the quantitative information they quote in their tables has had to be interpreted with great caution.

Nevertheless, they discussed three possible adverse effects of aluminium. These included neurologic damage in experimental animals and a possible relationship to Alzheimer's disease, aluminium retention in chronic renal failure, and bone disease as a result of phosphate depletion caused by aluminium hydroxide administration.

1.2.i CONCERN ABOUT ALUMINIUM OVERLOAD IN RENAL FAILURE

In the 1950's and 1960's, aluminium hydroxide was used world wide as a phosphate-binder in chronic renal failure. However, the debate about aluminium toxicity was started by the introduction of a new trial drug, an aluminium-phase resin for the control of hyperkalaemia(15).

Berlyne et al.(16) were the first to draw attention to aluminium-containing pharmaceuticals when they reported the finding of hyperaluminaemia in renal failure patients. The values were increased in some patients on oral treatment with aluminium-phase resins and aluminium hydroxide, and in some patients on dialysis treatment who were not taking oral aluminium salts, but who had been exposed to a dialysate with a relatively high aluminium content. Berlyne and co-workers proposed that the increased values in these patients were probably accounted for by considerable absorption of aluminium ions from the intestine and impairment of the normal efficient renal mechanism of excretion of aluminium.

Berlyne's team measured serum aluminium by neutron activation, colorimetric tests, and atomic absorption spectroscopy. However, their results were so far from those obtained today that they cannot be interpreted as confirming Berlyne's contention (though it eventually proved correct in all respects). The normal range of

aluminium concentration in serum was 20 - 100 times higher than the current figures and values for patients as high as 2000 and 4000 umol/l were reported (typical figures for patients consuming aluminium-containing medications today are in the range 1 - 15 umol/l).

They subsequently reported the effects of aluminium poisoning in uraemic and non-uraemic rats after modest doses of oral and parenteral aluminium salt(17).

Intra-peritoneal aluminium hydroxide was rapidly lethal to uraemic rats but not to controls. Oral aluminium sulphate and chloride were equally lethal to uraemic rats but not to controls. The intoxicated rats developed periorbital bleeding, corneal atrophy and heavy aluminium overload in liver, heart, striated muscle, brain and bone tissues.

It is understandable that Berlyne and his co-workers were alarmed by the lethality of aluminium in renal failure, but in view of the unusual routes of administration their recommendation "that pending further work, aluminium salts should not be prescribed for patients with renal functional impairment, and that they should be withdrawn from the market" was regarded as over-zealous. A lively controversy arose for a while although reports by Clarkson et al.(18) encouraged caution in the use of aluminium hydroxide.

After 1972, the debate on aluminium toxicity in the renal literature subsided and little was heard of the subject for the next four years. This was partly due to the lack of reliable methods of measuring aluminium in biological samples suitable for the detection of the very low concentrations of aluminium found in blood and tissues of normal subjects.

It was during these years that the first reliable methods were developed independently by Le Gendre and Alfrey,(19) and Fuchs et al(20). They described flameless atomic absorption spectrometric methods and were probably the first authors to show by reasonably accurate methods that phosphate-binders cause an elevation of serum aluminium.

It was also during these years that the two main clinical syndromes were described with Alfrey's account of encephalopathy (21) and Schorr's description of bone disease(22).

1.2.ii RECOGNITION OF ALUMINIUM INDUCED DISEASE

Dialysis encephalopathy

In 1972, Alfrey and his colleagues gave the first account of an outbreak of encephalopathy in a dialysis unit. They suggested that this was an intoxication and that the source of the toxin was the water used to make dialysis fluid(21). The first manifestation in these patients was a speech disorder, followed by dementia, convulsions and myoclonus. The syndrome was recognised in many other dialysis centres and considered to be a metabolic disorder of unknown aetiology.

By 1976 a sufficient number of papers describing this disease had been published for nephologists to accept it as a distinct entity(23). Alfrey and his colleagues subsequently proposed that this syndrome (dialysis encephalopathy or dialysis dementia), which developed after three to seven years of treatment, could be due to aluminium intoxication(24). Their proposal was based on the findings of increased aluminium content in brain, muscle and bone tissues in affected patients.

They proposed that increased tissue aluminium content in patients with the dialysis encephalopathy syndrome was derived from the oral aluminium hydroxide which had been administered therapeutically to control their serum

phosphate. In support of this proposal was the evidence of a positive aluminium balance in patients with chronic renal failure on haemodialysis treatment who were taking oral aluminium containing phosphate binding gels(18).

In spite of their earlier contention that the source of the toxin in dialysis encephalopathy was the dialysis fluid, when the evidence pointed to aluminium they were beguiled by the fact that their patients had been enthusiastically treated with aluminium hydroxide.

However, Alfrey's failure to mention the dialysate remained a puzzle and it seemed to have affected many other nephrologists with the same temporary blindness to the obvious.

The event which redirected attention to the dialysate was an outbreak of encephalopathy in one dialysis centre where the dialysis fluid was specifically shown to be the source of aluminium toxicity(25).

Following this, a survey (26) of water supplies throughout Great Britain showed an association between high tap water aluminium and the occurrence of encephalopathy so highly significant as to suggest that water aluminium was virtually the only important influence. Of course an association between water aluminium and encephalopathy does not constitute proof of causation.

Most of the existing evidence is now consistent with the notion that the excess brain tissue aluminium of dialysis encephalopathy syndrome stems from the dialysate with some contribution from the gastrointestinal tract.

That aluminium is the major toxic factor in the dialysis encephalopathy is now widely accepted (27)(28)(29). In an epidemiological analysis of six dialysis centres, using a uniform clinical classification, 55 patients with dialysis encephalopathy were identified (30). Dialysis encephalopathy was the direct cause of death in most cases, and the disease appeared to shorten survival significantly. The overall attack rate of dialysis encephalopathy was 4% and varied among the six centres from 2.2 to 14.7%.

The difference in the rates was explained by variations in aluminium exposure in the dialysate water. The risk of developing encephalopathy was significantly related to cumulative aluminium exposure in the dialysis water. It was also accepted that this syndrome had a common aetiology with one of the osteomalacic components of dialysis osteodystrophy (31)(32).

Hyperaluminaemia and dialysis encephalopathy may occur not only in patients on haemodialysis treatment but also in those on peritoneal dialysis,(33) and in some patients who have not been dialysed (34)(35). The non-dialysed

patients are usually children with renal failure who have been on oral treatment with aluminium hydroxide. Other workers have proposed that the aetiology of dialysis encephalopathy syndrome is multifactorial (36)(37). Even if this is so, aluminium undoubtedly has a major role.

Dialysis osteodystrophy

Whereas encephalopathy is a clear-cut syndrome most likely due to a single cause, aluminium bone disease merges into osteodystrophy of renal failure and the inter-relationship grows more complex as the literature expands. Bone pain, as a consequence of metabolic bone disease, is a common symptom in chronic renal failure patients on long-term intermittent haemodialysis. The progressive metabolic bone disease in these patients should be called 'dialysis osteodystrophy' a term that distinguishes it, and some aspects of its pathogenesis, from renal osteodystrophy in the undialysed patient.

The osteomalacic component of dialysis osteodystrophy is particularly troublesome in being associated with a high incidence of fractures. The reported incidence and rate of progression of the various components of dialysis osteodystrophy has varied not only between countries but also between dialysis centres within a country, despite apparently similar dialysis techniques (38)(39). The characteristic of dialysis osteodystrophy is that it

progresses or develops despite the maintenance of plasma calcium and magnesium, at concentrations which in a healthy person would not interfere with bone mineralization (40). In the bone mineralization process the availability of phosphate, for the formation of hydroxyapatite crystals, is of crucial importance. Phosphate deficiency, with hypophosphataemia, is a recognised cause of osteomalacia. The administration of aluminium-containing medications can induce a state of phosphate deficiency.

Attention was drawn to the direct role of aluminium in renal osteodystropy in 1971 when Parsons and co-workers reported an increased bone content of the element in patients with end-stage chronic renal failure (41). The aluminium content tended to be highest in patients who had been longest on haemodialysis. There was no correlation between the bone aluminium content and the amount of oral aluminium hydroxide that the patient had consumed. Although Parsons and his colleagues (41) did not at that time speculate on the potential sources of the aluminium, their observations could have been interpreted as pointing to the haemodialysis procedure itself.

In 1977 Platts et al. reported (42) on the prevalence of dialysis encephalopathy and spontaneous fractures in 202 patients on home dialysis for chronic renal failure. Noting the uneven geographical distribution of these

complications they investigated the water supplies. In the tap water of patients with fractures or encephalopathy, the concentrations of aluminium and manganese were higher than those in the water of patients without the complications. The patients with multiple fractures had been dialysed against water with higher aluminium and manganese content than those patients with a single fracture. Platts et al.(42) did not incriminate aluminium hydroxide ingestion in the genesis of these complications because only some of the patients took the gel, and even then, not consistently. Instead, they concluded that some contaminant in the water used for dialysis was very probably responsible for the development of dialysis encephalopathy and pathological fractures. Although they did not definitely incriminate aluminium as the toxic contaminant, they proposed that in areas with a high aluminium content in the tap water the patients should be dialysed with deionized water. The beneficial effect of using deionized water to prepare the dialysate was subsequently confirmed by other workers. Ward and his colleagues reported (43) that after one to four years of haemodialysis, osteomalacia was evident in only 15% of a group of patients using deionized water compared with 70% of a group using softened non-deionized water from the same source. In their dialysis centres which used tap water with a high aluminium content both osteomalacia and dialysis encephalopathy were encountered. The close association between these complications, in their

judgement, was consistent with a common aetiology. An epidemiological survey of 1,293 patients in 18 British dialysis centres showed a highly significant correlation between the aluminium content of water used to prepare the dialysate and the incidence of osteomalacic dialysis osteodystrophy and dialysis encephalopathy (26). The bulk of the evidence (44)(45)(46)(47) now supports the notion that aluminium is at least a very important aetiological factor in one type of osteomalacic dialysis osteodystrophy. The particular type of osteomalacia induced by aluminium is progressive, associated with a myopathy , and relatively resistant to treatment with either Vitamin D or its biologically active metabolites. In some series it has been associated with a high incidence of hypercalcaemia (48). The usual source of aluminium seems to be the tap water from which the dialysate is made. In addition there is undoubtedly some intestinal absorption from the phosphate-binding gels, and in some patients this seems to have been the dominant factor (35)(49). It is possible that individual aluminium absorption rates vary and that some patients may absorb excessive amounts from the intestinal tract after oral administration. The driving force for aluminium transfer during haemodialysis seems to be the effective concentration gradient between the dialysate aluminium and the free diffusible plasma aluminium (50).

The mechanism for the disordered bone formation induced by

an excess of aluminium remains to be determined. Aluminium was one of the polyvalent metal ions whose effect on the stability of a calcification buffer was studied by Bachra and Van Hanskamp (51). They reported that at concentrations of 1 umol/l (27 ug/l) or less aluminium ions destabilised the buffer, and they proposed that polyvalent ions such as aluminium, by formation of an insoluble phosphate, could initiate the precipitation of calcium apatite. This effect of aluminium in vivo would presumably interfere with the normal orderly deposition of bone mineral. In laboratory animals a striking increase of bone aluminium content with a disturbance of the mineralization process and the development of osteomalacia has been reported after intraperitoneal injection of aluminium chloride (52). The defect in mineralization did not arise immediately after the increase in bone aluminium, but after quite a long lag phase. Histologically the mineralization defect was characterised by an excess of osteoid. On the evidence of electron-probe X-ray microanalysis, and a specific histochemical stain, the aluminium in haemodialysis patients with osteomalacia, was mainly localised at the interface between the osteoid and the calcified matrix (48)(53)(54). The interface, or calcification front between the mineralised and non-mineralised matrix is normally the first site of bone mineral deposition. Cournot-Witner et al.(53) found no such localisation in patients with osteitis fibrosa and proposed that aluminium was

distributed diffusely throughout the bone tissue. These patients had higher plasma concentrations of immunoreactive parathyroid hormone, histologically increased osteoclast surface, and marrow fibrosis. Cournot Witmer et al.(53) concluded that osteomalacic dialysis osteodystrophy arises only in the absence of severe secondary hyperparathyroidism and proposed that the action of aluminium on bone cells and matrix mineralization may in some way be modulated by parathyroid hormone. The localisation of aluminium to the calcification front is encouraged by a low level of parathyroid activity, at least as judged by the effect of parathyroidectomy (55).

A reasonable, though not certain, interpretation of current data is that aluminium accumulation suppresses parathyroid activity, encouraging aluminium deposition at the ossification front and producing a vicious spiral of disease.

Microcytic anaemia

This is often the first clinical manifestation of aluminium intoxication and also the most readily reversible, so it is surprising that it was the last of the major syndromes to be recognised. Nearly all patients with chronic renal failure have a normochromic normocytic anaemia. The aetiology of the anaemia has several

components and it is not simply the end result of a decrease in renal production of erythropoietin (56). In 1978 Elliott and MacDougall reported (57) that anaemia was associated with osteomalacic dialysis osteodystrophy and with dialysis encephalopathy. In the encephalopathy patients the haemoglobin fell before the onset of neurological symptoms and in the osteodystrophy patients before bone complications. The mechanism by which an excess of aluminium induces anaemia remains to be determined.

Aluminium is deposited in bone marrow cells, particularly the reticular cells of the erythroblastic islets (58) where it presumably interferes with the accumulation of haemoglobin in developing red cells.

The anaemia caused by aluminium poisoning is non-iron deficient, microcytic and hypochromic, and responds to use of a low-aluminium dialysate (59)(60). In view of the nature of the anaemia induced by aluminium toxicity, O'Hare and Murnahagan (60) have proposed that it may be due to a disturbance in haem synthesis, specifically involving d-amino-laevulinic acid (ALA) dehydrogenase. Another possible explanation is that aluminium interferes with the transport of iron by competing for its binding sites on transferrin.

1.2.iii ALUMINIUM ACCUMULATION IN INDIVIDUALS WITH NORMAL RENAL FUNCTION

Further evidence of the harmful effects of aluminium comes from studies of aluminium accumulation in subjects who have normal renal function.

Oral aluminium

The widespread distribution of aluminium compounds in nature and their prevalence in water, foods such as infant formula feeds (61) and tea (62) as well as release from cooking utensils have stimulated considerable interest in the absorption of aluminium from the gastrointestinal tract. It has been estimated that the daily intake of aluminium in an average diet can vary from 2 to 160 mg (0.1 to 5.9 umol) (13)(14)(63).

In the early 1900's, a number of studies, both in man (64)(65)(66) and in experimental animals (66)(67)(68) were designed to investigate the absorption and excretion of aluminium. In the first systematic investigation (64) of this issue volunteers were fed biscuits or pancakes made from baking powder containing sodium aluminium sulphate. There was no significant increase in serum aluminium concentration detected and less than 0.1% of the ingested aluminium was excreted in the urine. Thus, it was concluded that aluminium is not readily absorbed through

the intestine and only trace amounts appear in tissues.
A number of modern studies have provided substantial evidence demonstrating some absorption of aluminium from the gastrointestinal tract (16)(69)(70)(71). Collectively these investigations indicate that the gastrointestinal absorption of aluminium is dependent on the dose as well as the chemical and physical form of aluminium administered. However, the precise relationship of the dose ingested to the dose absorbed is yet to be defined, although the best available data on this issue comes from balance studies (18)(64)(72)(73).

The data from the five balance studies were combined and more closely examined to investigate the relationship between the amount of aluminium ingested and the actual amount absorbed (74). Aluminium balance studies were completed in normal healthy subjects with careful monitoring of the aluminium ingested in the diet and from a variety of antacids. All faeces and urine were collected and analysed by atomic absorption spectroscopy. Absorption was defined as the difference between the dose administered and the concentration excreted in the faeces. A compilation of the data in 29 subjects is shown in Figure 1.1 which compares the dose of aluminium per day with the amount of aluminium absorbed per day. The levels of ingested aluminium cluster at three values, approximately 10 mg(0.3 mmol)/day,125 mg(4.6 mmol)/day, and approximately 2000 mg(74 mmol)/day. There was no

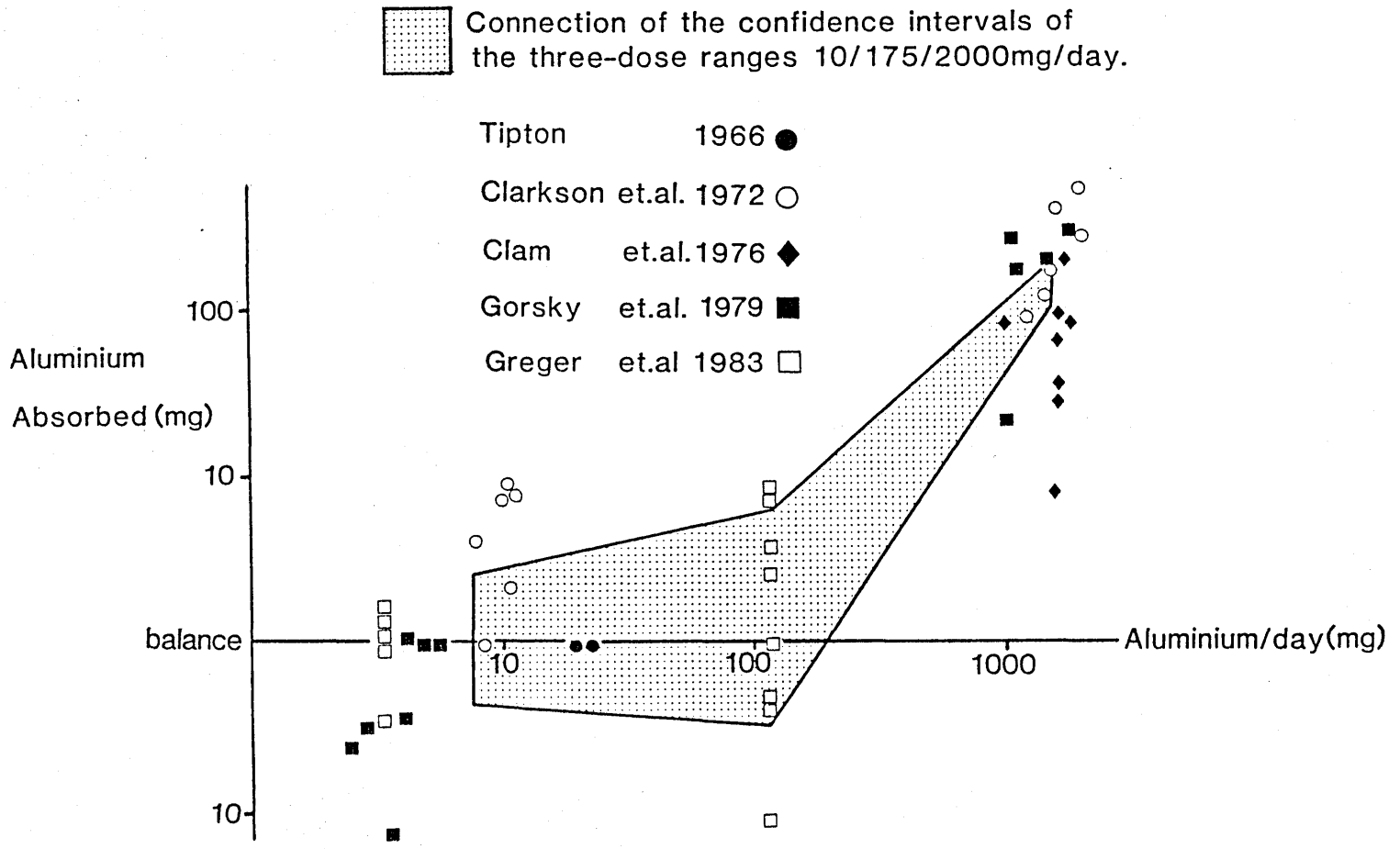

Figure 1.1 COMPARISON OF THE AMOUNT OF ALUMINIUM ABSORBED PER DAY VERSUS THE DOSE OF ALUMINIUM PER DAY
A compilation of data from five balance studies (29 subjects). Taken from Skalsky and Carchman (74)

appreciable absorption at the two lower doses. However, absorption does occur at the high (therapeutic) dose levels.

The precise prediction of the amount of aluminium absorbed for a given dose is not possible from this data. However, the simple connection of the confidence intervals of the three dose ranges provides an indication of the limits that such a dose-response curve might follow (Fig 1.1). Using this, it is possible to speculate that in a balance study no measurable absorption would be expected until the daily dose exceeded 225 mg(8.3 mmol)/day. The current evidence demonstrates that a dose-response relationship does exist for the gastrointestinal absorption of aluminium.

The absorbed aluminium that is not excreted could accumulate in tissues. Recker et al. (70) reported a patient who had chronic peptic ulcer disease and had been taking aluminium-containing antacids for 25 years. He had osteomalacia, and his bone aluminium was 24 ug/g. This was certainly higher than the normally accepted value of less than 8 ug/g,(75) but much lower than the levels seen in patients with renal failure.

There have been other reports of osteomalacia in normal individuals who ingest large quantities of aluminium-containing antacids.(76) It is not clear from these

studies whether the bone pathology was the result of aluminium accumulation or the effect of disturbance of mineral balance with hypophosphataemia and negative calcium balance.

Although the available evidence would suggest that there is negligible absorption of aluminium from the normal diet there has been considerable public concern (77)(78)(79) about the undesirable effects of dietary aluminium in food sources, e.g. tea, infant feeds and from cooking pots. However, there is little evidence to suggest that they are of more than marginal importance, contributing only a few milligramme per day to intake, compared with several grammes per day from phosphate-binders (80). The use of aluminium pans increase the aluminium content only slightly (81)(82). For example, it would take 20kg of cabbage soup boiled in an aluminium pan to equal one teaspoon of aluminium hydroxide. There are about 30mg (1.1 mmol) of aluminium in 80mg (3.0 mmol) of aluminium hydroxide (70) so a teaspoon of antacid will deliver about 125 (4.6)to 150mg (5.6 mmol).

A recent report (78) suggested that tea which contained up to 100 mg/l (3.7 mmol/l) of aluminium could be a very important source, but other studies (83)(84) show a maximum aluminium level of 4.9 mg/l (0.2 mmol/l) which is insignificant in comparison with medicinal doses.

The one food source which may be of significance is formula feeds for infants. Aluminium concentrations varying between 94 ug/l (3.5 umol/l) and 1330 ug/l (49.3 umol/l) were found in some milk formulae (61) and it was estimated that a typical infant would receive 17 (0.6) to 240 ug/kg (8.9 umol/kg) daily. Although this daily oral intake might not be regarded as excessive in adults, it may be potentially toxic to infants with immature or impaired renal function.

High urinary aluminium levels have been found in full-term infants with normal renal function suggesting increased gastrointestinal absorption at this age. In the presence of altered renal function which is common in pre-term infants, aluminium excretion may be reduced, and accumulation ensues.

Aluminium toxicity with increased brain aluminium has been reported in uraemic infants who were fed entirely with oral milk formulae contaminated with aluminium (85).

The tap water used for reconstituting feeds could also be contaminated with aluminium. In the North-West of England tap water aluminium concentrations range from 10 (0.4) to 300 ug/l (11 umol/l)(3). European Economic Community provisional regulations set the maximum aluminium concentration in drinking water at 200 ug/l (7.4 umol/l).

The potential toxicity of this source of oral aluminium to the general public has recently been highlighted in a survey of 88 county districts in England and Wales which have varying levels of aluminium in the water. The risk of Alzheimer's disease was 1.5 times higher in districts where the mean concentration of aluminium in the water exceeded 4 umol/l than in districts where concentrations were less than 0.4 umol/l. Subsequent correspondence disputes these findings and the role of aluminium in the aetiology of Alzheimer's disease (4)(5)(6)(7).

The extent to which aluminium is absorbed from the intestinal tract depends not only on the dose but also on its chemical form and further research is required to assess the bioavailability of aluminium from tap-water compared with other sources in the diet and to determine the fate of the absorbed aluminium within the body.

Parenteral aluminium:

intravenous nutrients

The parenteral route which bypasses the gastrointestinal barrier has proved a greater source of anxiety. Tissue accumulation of aluminium in patients with normal renal function due to the inadvertent adminstration of parenteral aluminium was first described in patients

receiving long term total parenteral nutrition. A syndrome of bone pain and fractures was found in patients from Los Angeles and Seattle (86)(87). They had a variety of gastrointestinal diseases with malabsorption. They had not been taking large amounts of antacids. The bone symptoms developed months to years after the administration of the total parenteral nutrition, at a time when the general nutritional status had improved. These patients had normal serum levels of calcium, phosphate, magnesium, bicarbonate, copper, zinc and albumin. The serum alkaline phosphatase activity tended to be higher than normal, but the parathormone levels were usually low, or in the lower part of the normal range. Vitamin D levels were normal, but 1,25 Dihydroxycholecalciferol was decreased. The patients had normal renal function with a mean creatinine clearance of 82 ml/min. The urine calcium was very high, especially during the total parenteral nutrition infusion, with a mean value of 456 mg (11.4 mmol) over 12 hours.

Bone biopsies from 14 out of 16 patients demonstrated poor bone formation as measured by double tetracycline labelling. Some of the patients had increased osteoid (osteomalacia) but others had normal or even decreased osteoid (aplastic bone). There was no fibrosis and the osteoblasts and osteoclasts did not appear active. Total bone area was also decreased. Aluminium stains were positive covering 4% to 77% of the bone surface. In the

other two patients no aluminium stain was seen. The cells appeared more active, and one had slight amounts of fibrosis. The bone formation was normal. The bone formation rate in this group of patients was significantly correlated with the bone aluminium concentration (88).

The patients with aluminium in the bone had been receiving intravenous nutrition with amino acids in the form of casein hydrolysate, whereas the other two patients had received synthetic crystalline amino acids. Further investigation of the total parenteral nutrition solutions revealed that the casein hydrolysate contained 2,313 $\pm$ 149 ug/l (85.7 $\pm$ 5.5 umol/l) of aluminium. The crystalline amino acids had only 26 ug/l (1 umol/l) and there were only small amounts in other fluids such as dextrose. These patients had been injected with 2(0.07) to 3.5 mg (0.13 mmol) of aluminium per day. Their plasma aluminium was 154 ug/l (5.7 umol/l) and bone aluminium ranged from 50 to 250 ug/g. Liver aluminium was also elevated at 84 ug/g (87).

Klein et al.(89)(90) did a series of studies in small groups of these patients to evaluate the aluminium kinetics. The plasma concentrations were measured after 10 hours of infusion. At rates of infusion of 2 ug (0.07 umol) of aluminium per minute there was no increase in the plasma level, suggesting rapid tissue uptake of the aluminium. The plasma levels did rise if the infusion

rate was 3 ug/min (0.11 umol/min). Urine aluminium averaged 1264 ug/day (46.8 umol/day) compared to the 15 ug/day (0.6 umol/day) seen in normal controls. The ultra-filterable aluminium was 5.5% of total serum aluminium, so that the calculated fractional excretion was 5% to 20%. The mean renal clearance of aluminium was 10.6 ml/min. The overall urine excretion was less than the load. In three patients balance studies were carried out. The faecal losses were small, only 5(0.2), 26(1.0) and 71 ug/day (2.6 umol/l). Thus, the net retention of aluminium was 438 (16.2) to 1,337 mg (49.5 umol) per infusion.

In both renal and total parenteral nutrition patients the parathormone and 1,25 Dihydroxycholecalciferol levels are low. These hormones may play a role in the pathophysiology of bone disease. It is possible that the aluminium itself may inhibit the parathyroid gland or the 1-α-hydroxylase in the kidney.

To study this, calcium was eliminated from the total parenteral nutrition solution until the patients serum calcium fell from a mean of 9.4 to 8.6 mg/dl. The parathormone increased from 9 $\pm$ 3 to 18 $\pm$ 8 pmol/l. This was significant as a group, but in some individuals there was no increase in parathormone. In two of these patients, 1,25 dihydroxycholecalciferol levels were measured and they remained undetectable despite a fall in calcium and an increase in parathormone. In two other

patients, phosphate was removed from the total parenteral nutrition solution. but the 1,25 dihydroxycholecalciferol levels did not increase. In another two patients, both calcium and phosphate were removed from the solution for 10 days and there was still no rise in the 1,25 dihydroxycholecalciferol levels. Thus, these patients with normal renal function had suppression of the 1,25 dihydroxycholecalciferol production, which was not dependent on serum parathormone, calcium or phosphate.

In a study of seven patients receiving total parenteral nutrition, De Vernejoul et al.(91) also found low bone formation. The patients had intermediate levels of serum aluminium of 52.4 $\pm$ 38.8 ug/l (1.9 $\pm$ 1.4 umol/l). The bone aluminium was not elevated in cortical biopsies (11.7 v 9.4 ug/g in patients with osteoporosis) and aluminium staining was negative. The 1,25 dihydroxycholecalciferol levels were normal and parathormone were normal in five and elevated in two.

In a recent study of patients on total parenteral nutrition that had not been contaminated with aluminium, Lipkin et al.(92) demonstrated a spectrum of disease. While the bone formation rates were decreased in patients with high or intermediate aluminium exposure, the bone formation in the patients without aluminium exposure were normal in four of six cases. Thus, total parenteral nutrition per se does not result in low bone formation.

Follow up studies in patients whose total parenteral nutrition solutions were changed to amino acids have shown that the patients symptoms improved within several weeks. In one patient the bone biopsy taken 3.5 years later still had a positive aluminium stain, but it had decreased from 77% to 17% of the surface. The tetracycline labels showed good formation in cortical bone, but not trabecula bone. After another year the bone formation was normal, and aluminium stain had vanished (88). Six other patients were studied one to two years after changing to amino acids, and bone biopsies were normal (93). Thus, when aluminium loading is discontinued, these patients with normal renal function can slowly eliminate aluminium, even from stores in bone.

The casein hydrolysate that was used in total parenteral nutrition patients contained about 2000 ug/l (74.1 umol/l) of aluminium. This is no longer used in clinical practice, but recently other intravenous solutions have been found to contain appreciable levels of aluminium. The total parenteral nutrition solutions used in France (88) had levels of 94 ug/l (3.5 umol/l) of aluminium with phosphate solutions containing up to 3645 ug/l (135 umol/l).

Sedman et al.(94) found that infants who had been receiving intravenous solutions had increased plasma aluminium levels of 36.7 ug/l (1.4 umol/l) compared with

5.2 ug/l (0.2 umol/l) in control infants and increased bone aluminium levels of 20.2 ug/g compared to 2.0 ug/g. Several components of the intravenous solutions were found to have high aluminium levels including phosphate salts, calcium salts, albumin and heparin. Similar levels of aluminium contamination of intravenous fluids have recently been reported with typical solutions for parenteral nutrition containing up to 3400 ug/l (126 umol/l) (61)(95).

albumin

Albumin was first implicated as a potential source of parenteral aluminium intoxication in 1978 when Elliott et al.(8) failed to treat dialysis encephalopathy by plasmapheresis because of unexpected aluminium contamination of the protein replacement solution. This important source of aluminium contamination was largely ignored at this time, presumably because of widespread interest in dialysate fluid contamination and its toxic effects on patients with renal failure.

Seven years later Milliner et al.(96) found a transient increase in plasma aluminium concentration and elevated urinary aluminium in a patient with normal renal function, undergoing plasma exchange with aluminium contaminated albumin. The patient received 1.2 mg of aluminium and his plasma aluminium concentration rose to 121 ug/l (4.5

umol/l), and then declined. The urinary aluminium excretion was 880 ug (33 umol) over 72 hours. There was no associated bone pathology. Several commercial preparations of albumin were screened and Milliner et al.(97) reported widespread contamination of albumin solutions. They suggested that the albumin solutions were contaminated during the manufacturing process.

Maher et al.(98) reported high plasma aluminium concentrations following intravenous albumin infusions in a patient receiving haemodialysis. They concluded that aluminium loading due to intravenous treatment was likely to be of particular importance in patients with renal impairment requiring prolonged parenteral nutrition, frequent albumin replacement treatment, or plasmapheresis.

Elevated plasma aluminium levels have been reported in patients receiving chronic intermittent plasma exchange (99) and also during single plasma exchange procedures (100). Thus, the potential toxicity particularly in patients with impaired renal function is again highlighted.

Contamination of albumin solutions with aluminium is now recognised in most products available throughout the world (97)(99)(101). Different sources of aluminium in albumin solutions have been implicated such as depth filters, filter aids, treatment of plasma with aluminium hydroxide

used for absorption of coagulation factors, and steel containers used during the manufacturing process (97)(102). Manufacturers of albumin solutions have until recently neglected this problem because of the lack of evidence of toxicity related to aluminium contaminated albumin.

1.2.iv MECHANISM OF ALUMINIUM TOXICITY

Neurotoxicity

The distribution of aluminium in serum and its binding by proteins has been investigated by a number of workers using a variety of techniques. The latter have included ultrafiltration,(103)(104) dialysis (50)(105)(106) and gel chromatography (107). The evidence available from these studies is consistent with the view that approximately 80% of aluminium in serum is protein-bound and appears to be associated with transferrin or possibly albumin (108) (109). The remaining 20% appear to be in the form of colloidal complexes. Citric acid and condensed phosphates in a pH range 2 - 6 form strong complexes with aluminium (110).

Transferrin is now considered to be the major aluminium transport protein (108)(109)(111) and it is probable that it plays a critical role in the delivery of aluminium to tissues such as bone and brain.

It is not clear why these tissues, and in particular brain, should be affected and this has been the subject of considerable research. It is possible that genetic defects may facilitate entry of aluminium into the brain. Another proposal (112) is that the extremely low aluminium flux into cells and across the blood-brain barrier may be the key. These processes depend on transferrin loading and transferrin receptor density, so that short-lived cells, unlike the long-lived neurones, may not be able to accumulate toxic levels of aluminium.

The mechanism by which aluminium acts as a neurotoxin has not been clearly defined. There are a number of potential sites at which aluminium may exert its neurotoxic action and these include protein synthesis, axonal transport and neurotransmitter related events (113). It has been demonstrated that aluminium acts as a neurotoxin by inhibition of dihydropteridine reductase resulting in a reduction of the brain content of tetrahydrobiopterin, tyrosine and neurotransmitters (114)(115). Conversely, it has been shown that reduction of aluminium in brain preparations from patients with Alzheimer's disease increases the synthesis of tetrahydrobiopterin (116).

The neurotoxicity of aluminium alternatively may involve alterations in the major post-synaptic enzymes of cholinergic neurotransmission.

It has also been reported that aluminium increases the permeability of the blood brain barrier to neuropeptides and to some non-peptide substances (117). In exerting this action aluminium appears to act directly to enhance the mechanism by which a substance normally penetrates the blood-brain barrier. It does not disrupt the barrier or increase permeability by enhancing pinocytosis or leakiness.

Bone disease

The type of osteomalacia that is associated with an excess of aluminium is unresponsive to treatment with either Vitamin D or its biologically active metabolites. The mechanism for the disordered bone formation induced by an excess of aluminium remains to be clarified. It may involve a disturbance either in the formation of calcium apatite crystals or in the actual bone mineralization process. It has been proposed that aluminium might have a different effect at varying bone concentrations with inhibition of mineralization at low concentrations and at higher concentrations a toxic effect on osteoblasts (118). There is also some evidence from in-vitro studies which suggests that aluminium may affect the activities of the bone enzymes acid and alkaline phosphatase and modify their response to parathyroid hormone and 1,25 dihydroxycholecalciferol (119).

Aluminium forms a complex with citrate that is a potent inhibitor of bone mineralization and retards the growth of calcium phosphate crystals in vitro. Thomas and Meyer (120) proposed that in patients with chronic renal failure the aluminium concentration reaches that which is required for the formation of the aluminium-citrate complex and that the action of the complex as a crystal poison could account for the failure of bone mineralization. Since albumin solutions contain the anticoagulant adenine-citrate-dextrose phosphate, the formation of the aluminium citrate complex is facilitated and this source of parenteral aluminium contamination may represent one of the most toxic forms of aluminium poisoning.

1.2.v PLASMA EXCHANGE

Plasma exchange was first performed by Fleig in 1910 (121) as a method of removing toxic substances from the blood. He used isotonic spa water as the replacement fluid and reported substantial clinical improvement. It is now common practice to infuse plasma or albumin to replace part or all of the lost plasma protein. The exchange of large volumes of plasma became possible with the introduction of cell separators. The first cell separator (122) consisted of a rapidly rotating conical vessel into which blood was drawn. The blood became separated into layers of red cells, leucocytes, platelets and plasma, so that in principle it was possible to harvest any particular fraction of the blood, although in practice satisfactory yields only of red cells or plasma could be obtained. Several modifications of this early design took place but the introduction of the continuous flow cell separator has formed the basis of present-day practice. In the IBM Separator,(123) blood is fed into a rapidly revolving bowl in which red cells, leucocytes, platelets and plasma separate into layers (figure 1.2). Any layer can be removed whilst the remainder of the blood is continuously returned to the donor. Most modern day cell separators have the advantage of using a totally disposable pathway and centrifuge bowl.

There are certain conditions in which plasma exchange has

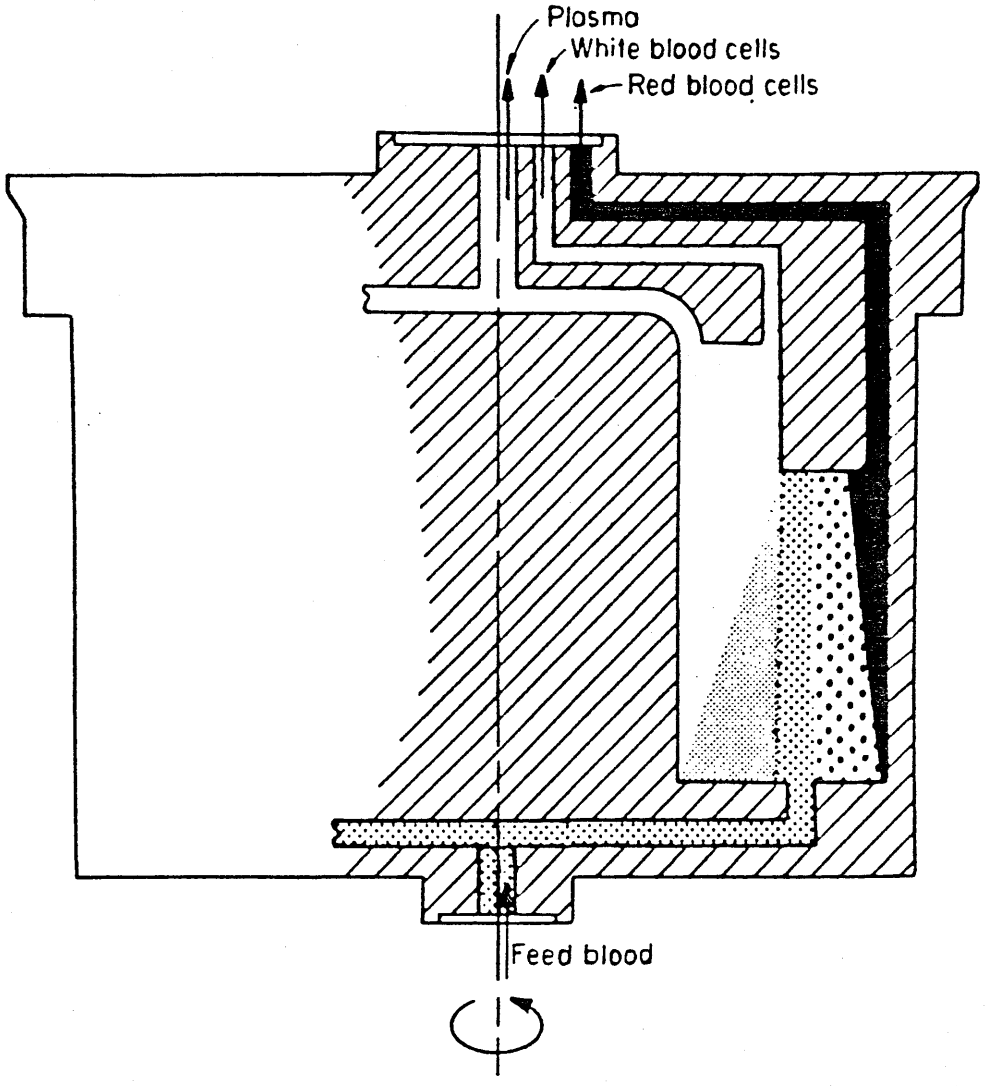

Figure 1.2 DIAGRAM OF A CONTINUOUS-FLOW BLOOD CELL SEPARATOR BOWL.

Blood is fed into the bottom of the motor and separates into layers, each of which can be collected continuously through the appropriate channel.

Taken from Mollinson.
(124)

been shown to be of definite benefit, namely the hyperviscosity syndrome, cryoglobulinaemia, myasthenia gravis, hypercholesterolaemia, and thrombotic thrombocytopenic purpura. There are also many conditions in which suggestive evidence of the value of plasma exchange has been obtained but in which it will be impossible to reach definite conclusions until controlled trials of treatment have been carried out. These conditions may be considered under the following headings (124).

1. Removal of antibodies

a) Alloantibodies, e.g. anti-Rhesus in pregnant women.
b) Autoantibodies, e.g. myasthenia gravis, Goodpastures syndrome.

2. Removal of inflammatory mediators.

3. Removal of exogenous toxins, e.g. paraquat poisoning.

4. Removal of excess plasma constituents, e.g. paraproteins, low density lipoproteins.

Since albumin is used as the replacement fluid, a previously unrecognised hazard of plasma exchange in these conditions is aluminium toxicity particularly in those patients on chronic intermittent plasma exchange. This

risk is greater in patients with impaired renal function.

Plasma exchange has been shown to be of value in patients with rapidly advancing renal failure due to crescentic nephritis caused by anti-glomerular basement membrane antibodies(125) or by immune complexes (126) and in chronic forms of progressive glomerulonephritis such as mesangiocapillary glomerulonephritis (MCGN) or idiopathic membranous nephropathy (IMN) (127). Since these patients may receive plasma exchange for periods varying from several months to years (125)(127) it is possible that considerable loading of parenteral aluminium via albumin solutions occurs. Features of aluminium toxicity have not been previously described in this group of patients.

CHAPTER 2.

METHODS

ALUMINIUM AND TRACE METAL ASSAY

General considerations

Aluminium, Chromium and Manganese measurements

Electrothermal atomic absorption spectrometry

Aluminium:

calibration

sensitivity

accuracy and imprecision

Chromium and Manganese:

calibration

sensitivity

accuracy and imprecision

Trace metal measurements

Inductively coupled argon mass spectrometry

Nickel, Manganese, Iron, Zinc, Copper, Selenium:

calibration

sensitivity

accuracy and imprecision

Sample collection

Plasma

Urine

Bone

Albumin

2.1 ALUMINIUM AND TRACE METAL ASSAY

2.1.i GENERAL CONSIDERATIONS

Clinical and other biochemical studies of aluminium and trace metals are dependent on the availability of accurate and precise analytical methods. All biological specimens are complex mixtures of a multitude of organic and inorganic constituents, making the detection of a true constituent, such as aluminium extremely challenging.

During the last decade reports of aluminium toxicity have lead to a search for a sensitive, reliable and simple method to measure aluminium in biological samples, both at toxic and physiological concentrations. Applicable methods include qualitative chemical staining techniques,(128)(129) X-ray microanalysis,(50)(130)(131) secondary-ion mass spectroscopy and laser microprobe mass analysis,(132) quantitive neutron activation, (133) (134) (135) inductively coupled plasma atomic emission spectrometry (136)(137) and electrothermal furnace atomic absorption spectrometry. (138)(139)(140) The latter two single element methods have also been modified to provide multi-element profiles of biological fluids (141).

In this study the method used for the quantitative determination of aluminium, chromium and manganese was electrothermal furnace atomic absorption

spectrophotometry, while simultaneous multi-element trace analysis of nickel, iron, magnesium, zinc, copper and selenium was determined by inductively coupled argon plasma mass spectrometry (141). These methods offer the best sensitivity, selectivity and simplicity.

2.1.ii ALUMINIUM, CHROMIUM AND MANGANESE MEASUREMENTS

Electrothermal atomic absorption spectrometry

In this technique the sample is placed in a graphite tube furnace (carbon rod) mounted in the light path of the spectrophotometer. The source of light is an aluminium, chromium or manganese hollow cathode lamp which emits a characteristic wavelength. First the graphite tube is heated with a direct current to dry the sample at a low temperature (80 C to 300 °C) then the sample is ashed (700 °C to 1300 °C) to destroy organic matter or matrices and burn off inorganic species that may interfere and finally the temperature is quickly raised to 2,750 °C and the metal under analysis vaporises and absorbs the light being passed through the graphite tube. The absorption signal is a sharp peak, the height or area of which can be related to the amount of metal present. Microlitre (2 to 100 ul) samples are introduced into the furnace by injection with an auto-sampler and the sample is dried, ashed and atomised.

Aluminium:

Aluminium assays on plasma, urine, albumin, other blood products and bone were carried out by electrothermal atomic absorption spectrometry on a Perkin- Elmer (PE) 2280 spectrometer, equipped with a Perkin-Elmer HGA 500 Carbon furnace and AS-1 auto sampler with a model 56 recorder as detailed by Gardiner et al.(138).

calibration

Calibration was carried out using aqueous standards and this has been reported as adequate for the determination of aluminium in plasma, serum and urine (138)(139)(140).

sensitivity

The detection limit as measured by the signal noise obtained from a 2.2 umol/l aqueous standard was 0.13 umol/l.

accuracy and imprecision

"In-house" pooled samples of plasma, urine and bone were used as internal quality control. Plasma samples circulated by the Robens Institute, The University of Surrey were used as external quality control checks.

Plasma spiked with aluminium (+3.7 and +7.4 umol/l) showed a mean recovery of 95%. Imprecision was evaluated by monitoring variations between repeated measurements and the coefficient of variation was 2.9% at a concentration of 10 umol/l.

Chromium and Manganese:

Manganese and Chromium assays on plasma, urine albumin and other blood products were carried out by electrothermal atomic absorption spectrometry using the same instrumentation as for aluminium.

calibration

Calibration was carried out using aqueous standards (142)(143).

sensitivity, accuracy and imprecision

Manganese : Detection limit was 2 nmol/l.
Coefficient of variation was 3.7% at a concentration of 84 nmol/l.

Chromium : Detection limit was 10 nmol/l.
Coefficient of variation was 6.1% at a concentration of 38 nmol/l.

The accuracy of the procedure was assured by a satisfactory interlaboratory comparison of pooled 4.3% human albumin solution (141).

2.1.iii TRACE METAL MEASUREMENTS

Inductively coupled argon plasma mass spectrometry

This new method has only relatively recently been applied to clinical samples. The instrument is a combination of two well-proven techniques in which a high temperature argon plasma (8000 °C) is produced by inductive electrical heating. Liquid samples are sprayed into the plasma via a nebulizer.

Rapid heating dissociates all compounds and produces ions for each element present. These ions are collected from the central plasma through a special aperture into a quadraple mass analyser, in which the number of counts for each ionic mass is measured.

Nickel, Manganese, Iron, Zinc, Copper and Selenium:

Nickel, Magnesium, Iron, Zinc, Copper and Selenium assays were obtained using a Plasma Quad (VG Elemental, Winsford, Cheshire, UK).

calibration

Calibration in a semi-quantitative scan mode was carried out using Indium (25 ng/ml) as an internal standard or by use of appropriate multi-element aqueous standards (141).

sensitivity

The detection limit was less than 10 nmol/l for all elements assayed.

accuracy and imprecision

A within-batch imprecision of less than 5% and a between-batch imprecision of less than 10% was obtained for all the metals as reported by Lyon et al (141). Accuracy has been shown to be good by use of external reference materials and direct comparison of results with established atomic absorption spectrometric methods (141).

2.1.iv SAMPLE COLLECTION

Sample contamination is a serious problem because of the ubiquitous nature of aluminium and trace metals and great care was taken with collection as follows:

Plasma

Blood was collected after venepuncture with a stainless steel needle into a plastic syringe via a venflon plastic cannula. The samples were then immediately transferred to an aluminium-free plastic tube with sodium citrate as the anticoagulant. Plasma was separated within 20 - 60 minutes and aliquoted into aluminium-free plastic containers which were stored at 4^{o}-C. prior to analysis.

Urine

Twenty-four hour urine specimens were collected in aluminium-free plastic containers which had previously been cleaned with nitric acid. A 10 ml aliquot was transferred to a sterile polypropylene tube which was stored at 4°C.

Bone

Samples for aluminium analysis were placed in an aluminium-free plastic container and stored at room temperature. Prior to analysis the bone was dissolved in nitric acid.

Albumin

2ml aliquots were removed from each bottle of 5% or 20% albumin solution or blood product using a stainless steel needle and plastic syringe. This was immediately transferred to an aluminium-free plastic container and stored at 4°C. prior to analysis.

CHAPTER 3.

DETERMINATION OF THE DEGREE OF ALUMINIUM AND TRACE METAL CONTAMINATION OF ALBUMIN SOLUTIONS AND BLOOD PRODUCTS ISSUED FOR TREATMENT OF PATIENTS

STUDY DESIGN

Aluminium

Trace metals

RESULTS

DISCUSSION

3.1 STUDY DESIGN

Aluminium

Samples were taken from at least three batches (range 3 - 10) of all the available blood products which were present in the Blood Transfusion Laboratory of the Royal Infirmary, Glasgow.

The majority of the products were manufactured by the Protein Fractionation Centre of the Scottish National Blood Transfusion Service. Other manufacturers whose products were tested include Armour Pharmaceuticals, Speywood Laboratories, Wrexham, Immuno A.G. Vienna, Austria, Hoechst UK and the Protein Fractionation Laboratory, Oxford.

Aluminium concentrations were determined by electrothermal atomic absorption spectrometry using a method developed for measurement of plasma aluminium (138). The blood products studied were albumin solutions (5% and 20%) from three different manufacturers, fresh frozen plasma, cryoprecipitate, human factor IX Concentrate, anti-haemophilic factor VIII, porcine factor VIII, anti-inhibitor coagulant complex (F.E.I.B.A.) human immunoglobin preparations (for intravenous and intramuscular use) and antithrombin III concentrates.

Trace Metal Contamination of Albumin Solutions and Blood Products

Using electrothermal atomic absorption spectrometry chromium and manganese concentrations were determined in blood products produced by four manufacturers (Table 3.4). Inductively coupled argon plasma mass spectrometry was used to determine the concentrations of nickel, iron, magnesium, zinc, copper and selenium in samples of single batches of albumin solutions (5% and 20%) from three different manufacturers (Immuno, Scottish National Blood Transfusion Service, Armour).

3.2. RESULTS

All of the albumin solutions contained significant amounts of aluminium compared to normal plasma (aluminium <0.5 umol/l). The concentrated albumin solutions (20%) contained more aluminium (Immuno 46.5 ± 1.9 umol/l, Scottish National Blood Transfusion Service (SNBTS) 30.5 ± 1.5 umol/l, Armour Pharmaceuticals 20.4 ± 1.2 umol/l) than 4.3% albumin solutions (Immuno 9.1 ± 1.4 umol/l Scottish National Blood Transfusion Service 18.3 ± 2 umol/l Armour pharmaceuticals 6.7 ± 1.4 umol/l) see Table 3.1. All the factor VIII concentrates and immunoglobulin preparations contained significant aluminium levels whereas human factor IX and antithrombin III concentrates contained aluminium levels within the normal range (Table 3.2).

Increases in concentration of ten fold to several thousand fold the normal reference values were noted for chromium, manganese, nickel and iron. There were also marked depletions of essential trace elements such as magnesium, zinc, copper and selenium (Table 3.3, Table 3.4).

Table 3.1 ALUMINIUM CONTENT OF ALBUMIN AND COLLOID SOLUTIONS

SUPPLIER/MANUFACTURER	PRODUCT	NO. OF BATCHES	NO. OF SAMPLES PER BATCH	ALUMINIUM MEAN + SD µmol/L
ALBUMIN SOLUTIONS				
IMMUNO A.G. VIENNA, AUSTRIA	4.3% PLASMA PROTEIN FACTOR	8	10	9.1 ± 0.6
IMMUNO A.G. VIENNA, AUSTRIA	20% HUMAN ALBUMIN	3	10	46.5 ± 1.9
S.N.B.T.S. *	4.3% STABLE PLASMA PROTEIN SOLUTION	7	10	18.3 ± 2.0
S.N.B.T.S. *	20% HUMAN ALBUMIN	3	10	30.5 ± 1.5
ARMOUR PHARMACEUTICALS	5% HUMAN ALBUMIN	3	10	6.7 ± 1.4
ARMOUR	20% HUMAN	3	10	20.4 ± 1.2
COLLOID SOLUTIONS				
BEHRINGWERKE A.G. MARBURG FRG	HAEMACEL	3	10	0.7

* (Protein Fractionation Centre, Edinburgh
(SCOTTISH NATIONAL BLOOD TRANSFUSION SERVICE

NORMAL REFERENCE RANGE FOR ALUMINIUM <0.5 umol/L

Table 3.2 ALUMINIUM CONTENT OF BLOOD PRODUCTS

SUPPLIER/MANUFACTURER	PRODUCT	NO. OF BATCHES	NO. OF SAMPLES PER BATCH	ALUMINIUM MEAN + SD µmol/L
W.S.B.T.S. + CARLUKE	FRESH FROZEN PLASMA	3	10	0.1
W.S.B.T.S. + CARLUKE	CRYOPRECIPITATE	3	10	0.2
S.N.B.T.S. *	HUMAN FACTOR IX CONCENTRATE	3	5	1.6 ± 0.1
S.N.B.T.S. *	ANTI-HAEMOPHILIC FACTOR FACTOR VIII (HUMAN)	3	10	43.4 ± 2.3
SPEYWOOD LABORATORIES WREXHAM	HYATE 'C' FACTOR VIII (PORCINE)	3	10	7.7 ± 0.9

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Table 3.2 ALUMINIUM CONTENT OF BLOOD PRODUCTS

SUPPLIER/MANUFACTURER	PRODUCT	NO. OF BATCHES	NO. OF SAMPLES PER BATCH	ALUMINIUM MEAN + SD umol/L
IMMUNO A.G. VIENNA, AUSTRIA	F.E.I.B.A. ANTI INHIBITOR-COAGULANT COMPLEX	3	10	14.1 ± 1.2
S.N.B.T.S. *	HUMAN NORMAL IMMUNOGLOBULIN (INTRAVENOUS)	3	5	7.2 ± 0.6
S.N.B.T.S. *	HUMAN TETANUS IMMUNOGLOBULIN	3	3	20.8 ± 1.5
S.N.B.T.S. *	HUMAN IMMUNOGLOBULIN (IgG)	3	3	23.3 ± 2.0
PROTEIN FRACTIONATION LABORATORY, OXFORD	ANTI-THROMBIN III	3	3	0.1

* (Protein Fractionation Centre
(SCOTTISH NATIONAL BLOOD TRANSFUSION SERVICE

Table 3.3 - TRACE METAL CONTENT OF ALBUMIN SOLUTIONS USED FOR INTRAVENOUS INJECTION

PRODUCT SUPPLIER/MANUFACTURER	Al umol/l	Cr nmol/l	Fe umol/l	Mn nmol/l	Cu umol/l	Zn umol/l	Mg nmol/l	Se umol/l	Ni nmol/l
ALBUMIN 4.3% IMMUNO A.G. VIENNA, AUSTRIA	11	82	17	90	7.0	6.0	0.38	0.47	68
ALBUMIN 4.3% SCOTTISH BLOOD TRANSFUSION SERVICE, EDINBURGH, U.K.	19	2500	175	6000	5.3	3.8	0.61	0.22	8800
ALBUMIN 5% ARMOUR PHARMACEUTICAL CO. U.S.A.	7	54	17	82	2.5	6.5	<0.1	0.47	184
HAEMACEL COLLOID SOLUTION BEHRINGWERKE A.G. MARBURG FRG>	0.7	1200	<1	400	1.0	<1.0	<1.0	<0.05	100
"NORMAL PLASMA" REFERENCE RANGE	<0.5	<5	8-30	7-27	11-23	12-18	0.7-1.0	0.8-2.0	<10

Table 3.4 CHROMIUM AND MANGANESE CONTENT OF BLOOD PRODUCTS

SUPPLIER/MANUFACTURER	PRODUCT	CHROMIUM nmol/L	MANGANESE nmol/L
SPEYWOOD LABORATORIES WREXHAM	HYATE 'C'	160	205
IMMUNO AG VIENNA AUSTRIA	F.E.I.B.A.	119	401
S.N.B.T.S. *	lg- NORMAL	83	619
S.N.B.T.S. *	lg-HUMAN TETANUS	89	428
S.N.B.T.S. *	lg - ANTI-RH	140	637
PROTEIN FRACTIONATION LABORATORY, OXFORD	ANTITHROMBIN	36	198
REFERENCE VALUES		<5	7-27

Protein Fractionation Centre

* SCOTTISH NATIONAL BLOOD TRANSFUSION SERVICE

3.3 DISCUSSION

There was significant contamination of all the albumin products with aluminium. Similar levels of aluminium contamination were found by several authors when most of the products currently available were analysed (94) (95) (97) (98) (99). The aluminium concentrations in products from a single supplier were similar and this suggests that each manufacturing process might have its own specific source of aluminium. There is little doubt that metal contamination occurs during the production cycle since human plasma, which is the starting material, is low in aluminium. In addition there is no evidence for aluminium contamination from aluminium bottle caps or from distilled water from distillation and sterilization plants (144).

Other blood products including coagulation factors such as factor VIII, F.E.I.B.A. (anti-inhibitor coagulant complex) as well as immunoglobulin preparations have similar concentrations of aluminium contaminations to albumin solutions. These other potential sources of aluminium toxicity seem to be little recognised since there are no clinical observations of acute or chronic aluminium toxicity reported in spite of the widespread use of these blood products. A possible explanation is that the majority of patients, namely haemophiliacs, who receive these products have normal renal function and therefore excrete most of the aluminium. Additionally the volume of

product infused is lower. However, the long term neurotoxic effects of exposure to aluminium remains a potential problem, especially for those with some degree of renal involvement.

Apart from aluminium there is a widely varying trace metal contamination of albumin and colloid solutions. When the results for various protein products are compared with the normal plasma reference range, increases in concentration 10-fold to several 1000-fold are noted for chromium, manganese, nickel and iron. There are marked depletions of essential trace metals such as magnesium, copper, zinc and selenium.

Manufacturers and clinicians were unaware of this problem until it was reported recently (101). It is not uncommon for large volumes of albumin and colloid solutions to be used in the treatment of patients with renal impairment who are not able to excrete the infused load of potentially toxic metals. The toxicity of aluminium is now well established, but the clinical significance of the repeated infusion of small amounts of other metals is at present quite unknown. In a survey of several albumin preparations Leach et al. (145) noted considerable nickel contamination and pointed out various potential adverse effects, including exacerbation of allergies. It is possible that a reaction to trace metals, such as nickel, might explain the attacks of rigors, shivering and

hypotension which some patients experienced while undergoing plasma exchange, with the most heavily contaminated albumin solutions. It is interesting that these reactions occurred more frequently when contaminated Scottish National Blood Transfusion albumin was used as the replacement fluid, whereas the Immuno albumin and Haemaccel solutions did not appear to cause these problems. These clinical observations have also been made by other clinicians who treat patients with regular plasma exchange (146) and this matter warrants further investigation.

In addition to problems which exist with aluminium contamination of albumin and colloid solutions, there are also marked depletions of trace elements, such as magnesium, zinc, copper and selenium. An acute decrease of serum copper, selenium and zinc concentrations was reported immediately and found to last for several weeks following plasma exchanges with albumin solutions (147)(148). There were no symptoms suggesting plasma exchange-induced trace element deficiency in these patients. However in certain clinical situations where plasma exchange may be repeated once or twice per month for several years, chronic depletion of body stores of these trace elements may occur. Recent reports indicate an association between selenium deficiency and an increased incidence of coronary artery disease, and those patients on long-term plasma exchange may be at risk

(149). The losses during plasma exchange are obviously related to the elemental composition and content of the replacement fluid and might be controlled by using fluids where trace elements are present in similar concentrations and in the same chemical form as in normal plasma.

CHAPTER 4.

ALUMINIUM RETENTION AND ALUMINIUM BONE DISEASE IN PATIENTS RECEIVING PLASMA EXCHANGE WITH CONTAMINATED ALBUMIN

STUDY DESIGN

Patients

Plasma exchange regimen

Metabolic balance study

Calculations

- Total aluminium output
- Aluminium removed in plasma during exchange
- Aluminium excreted in urine
- Recovery of infused aluminium
- Faecal aluminium

Detection of patients with bone aluminium overload

- Bone biopsies
- Histomorphometric assessment:
  - bone resorption and formation
  - mineralization
- Desferrioxamine mobilization test

RESULTS

Albumin

Balance studies

Plasma aluminium concentrations

Histomorphometry and bone aluminium content

Desferrioxamine mobilization test

DISCUSSION

4.1. STUDY DESIGN

4.1.i PATIENTS

Eight patients (five males, three females), aged 21 to 74 years, undergoing regular plasma exchange (two months to seven years) for a variety of disorders were studied (Table 4.1). Two patients had mesangiocapillary glomerulonephritis and one had IgA nephropathy. Three had Waldenstrom's macroglobulinaemia, one had Guillain-Barre Syndrome and one had myasthenia gravis. Patients were divided into two groups, those with poor renal function, Group 1 (Creatinine Clearance < 50 ml/min and those with adequate renal function, Group II (Creatinine Clearance > 50ml/min) (Table 4.2). Two of the patients (MC, TS) in Group 1 had suffered intermittent bone pain while receiving plasma exchange.

4.1.ii PLASMA EXCHANGE REGIMEN

On each occasion 2.8 litres of albumin solution were exchanged. All Group 1 patients received 4.3% Human Albumin (Immuno AG Vienna, Austria), during the exchange procedure which was carried out using an IBM Continuous Flow Cell Separator. Group II patients were plasma exchanged with 4.3% albumin solution (Protein Fractionation Centre, Scottish National Blood Transfusion Service, Edinburgh, UK), using a Fenwall Continuous Flow

Table 4.1 CLINICAL DETAILS OF PATIENTS HAVING PLASMA EXCHANGE AND CALCULATED LOAD OF ALUMINIUM INFUSED

CASE	SEX AND AGE (YEARS)	DIAGNOSIS	TIME ON PLASMA EXCHANGE	TOTAL NO. OF PLASMA EXCHANGES	TOTAL VOLUME OF ALBUMIN RECEIVED (l)	CALCULATED INPUT OF ALUMINIUM (μmol/L)
MC	F 23	MESANGIOCAPILLARY GLOMERULONEPHRITIS	4 YEARS 5 MONTHS	119	274	2983
JM	M 21	IgA NEPHROPATHY	6 MONTHS	29	82	885
TS	M 45	MESANGIOCAPILLARY GLOMERULONEPHRITIS	7 YEARS	187	524	5707
SS	F 64	WALDENSTROM'S MACROGLOBULINAEMIA	4 YEARS	12	32	291
IM	M 52	WALDENSTROM'S MACROGLOBULINAEMIA	2 YEARS 3 MONTHS	60	144	1570
SW	M 61	MYASTHENIA GRAVIS	4 YEARS 6 MONTHS	11	31	282
HC	M 74	WALDENSTROM'S MACROGLOBULINAEMIA	2 YEARS	5	15	275
EA	F 52	GUILLAIN-BARRE SYNDROME	2 MONTHS	5	15	270

Cell Separator.

4.1.iii METABOLIC BALANCE STUDY

During each plasma exchange, samples of all the replacement albumin solutions infused and all plasma removed from the patients were analysed for aluminium. In patients undergoing a single plasma exchange, plasma aluminium concentration was measured before exchange and at 0, 24, 48 and 72 hours. Twenty-four hour urinary aluminium excretion was measured before and for three consecutive days after the procedure.

In those patients receiving 2 or 3 consecutive plasma exchanges, additional measurements were made of plasma aluminium concentration before and after completion of each exchange, and twenty-four hour urinary aluminium output was measured following each exchange.

4.1.iv CALCULATIONS

Total Aluminium Output

This was derived from the known volume of albumin infused at each exchange or series of exchanges, and the measured aluminium concentration of that solution.

Aluminium Removed in Plasma During Exchange

This was derived from the volume of plasma removed from the patient and the measured aluminium concentration of that solution. This quantity was then expressed as a percentage of the total infused aluminium load.

Aluminium Excreted in Urine

The daily output of aluminium was calculated from the urine volume and the measured urinary aluminium concentration. The amount excreted over the three day period was then determined and from that total three times the pre-exchange daily urine aluminium output was subtracted to allow for base-line urine aluminium excretion. The net urine aluminium excretion was then expressed as a percentage of the total infused aluminium load.

Recovery of Infused Aluminium

This is the sum of the aluminium excreted in the urine (3 days) and the aluminium removed in plasma, expressed as a percentage of the total infused load.

Faecal Aluminium

Faecal aluminium was not measured in the balance studies because only a very small proportion of an injected dose of aluminium would be normally be excreted in the faeces (74) (150).

4.1.v DETECTION OF PATIENTS WITH BONE ALUMINIUM OVERLOAD

Bone Biopsies

Four patients (MC, JM, TS - Group I and IM - Group II), each underwent two bone biopsies of the anterior iliac crest after tetracycline double labelling. Bone biopsies were performed using a Meuniere needle providing a core of 8mm in diameter. On the first biopsy histomorphometric analysis of undecalcified sections was performed after fixation in four percent neutral buffered formaldehyde and embedding in methacrylate (48). The presence of aluminium in bone was shown by a staining procedure using aurine tricaboxylic acid (Aluminon). The bone aluminium content (ug/g dry weight) was determined on the second bone biopsy from each patient.

Histomorphometric Assessment:

bone resorption and formation

This was assessed with a Zeiss One integrating eye piece, by line intersect measurement of total osteoid surface (TOS), active osteoid surface (AOS), total resorption surface (TRS), an active resorption surface (ARS) on four representative eight micrometer sections stained with a 1% toluidine blue (48). These measurements gave an indication of the degree of secondary hyperparathyroidism.

mineralisation

This was assessed by measuring the extent of calcification fronts along the osteoid surfaces on four representative stained eight micrometre sections (1% toluidine blue, 5% (ETA) and by counting the maximum number of bright osteoid lamellae (MNOL) visualised with polarising microscopy.

Desferrioxamine Mobilization Test

To further demonstrate the extent of aluminium retention a desferrioxamine infusion was given to five patients (MC, JM, TS, Group I and IM, SW, Group II) (151). Four grammes of desferrioxamine were infused in 250ml of 5% dextrose over a four hour period. This was carried out at least 2 weeks after the last plasma exchange. Plasma

aluminium concentrations were measured before, and 48 hours after the infusion of desferrioxamine. The 24 hour excretion of aluminium in urine was measured before and for three consecutive days after the infusion.

The amount of aluminium excreted in urine over those three days following the tests was calculated and three times the pre-test urine aluminium daily output subtracted to give a net urine aluminium mobilised (Appendix II).

4.2. RESULTS

4.2.i ALBUMIN SOLUTIONS

Each of the albumin solutions tested contained a high concentration of aluminium relative to normal plasma (<0.5 umol/l). Albumin produced by the Scottish Blood Transfusion Service Protein Fractionation Centre contained twice as much aluminium (18.3 umol/l) as Immuno human albumin (9.1 umol/l) (Table 3.1).

4.2.ii BALANCE STUDIES

Patients received an intravenous load of aluminium which varied from 11 umol to 123 umol (Table 4.2). Three patients (MC, JM and IM) were each studied on two occasions separated by one month. Patient IM received a similar load of aluminium on each occasion, while patients MC and JM received different loads. The proportion of aluminium excreted in the urine, that removed during plasma exchange, and the overall retention were similar for each patient on the two occasions.

The total amount of aluminium retained by the patients was dependent upon their renal function. As can be seen in Table 4.2, those with a creatinine clearance of less than 50 ml/min retained 60-74% of the infused aluminium. In Group II (creatinine clearance >50 ml/min) those patients with a creatinine clearance of greater than 70 ml/min

Table 4.2 - METABOLIC BALANCE STUDIES: ALUMINIUM

CASE	CREATININE CLEARANCE (ml/min)	ALBUMIN INFUSED IN ALBUMIN (umol)	ALUMINIUM EXCRETED IN URINE OVER THREE DAYS (umol)	ALUMINIUM REMOVED DURING PLASMA EXCHANGE (umol)	TOTAL AMOUNT OF ALUMINIUM RETAINED AS % OF INFUSED LOAD
MC*	11	23.8	5.0	4.4	60.6
MC*	10	36.4	5.9	5.4	69.0
JM*	11	39.2	5.3	3.9	67.5
JM*	10	109.0	13.0	15.4	74.0
TS	8	30.8	3.5	5.5	70.6
SS	27	11.0	2.8	0.9	66.0
IM*	55	55.2	28.1	4.2	42.0
IM*	51	54.0	19.0	2.7	60.0
SW	71	69.6	59.2	7.9	3.6
HC	91	123.0	122.0	5.3	0
EA	121	96.0	85.0	5.3	6.5

* Patients studied on two occasions (interval one month)

only retained upto 6% of the infused aluminium load.

The influence of deteriorating renal function on the urinary output of aluminium is illustrated in Figure 4.1 which also shows that the amount of aluminium removed in the "plasma bag" was a small proportion of the input.

4.2.iii PLASMA ALUMINIUM CONCENTRATIONS

The patients with a creatinine clearance of less than 50 ml/min had a tendency for their plasma aluminium concentrations after exchange to be higher than those with adequate renal function (Table 4.3).

However plasma aluminium concentration did not reflect the extent of aluminium retention in any given patient.

4.2.iv HISTOMORPHOMETRY AND BONE ALUMINIUM CONTENT

The three patients with poor renal function had raised concentrations of aluminium in their bone biopsy specimens. (Reference value < 10ug/g dry weight bone) (Table 4.4).

Patient MC, after 53 months on plasma exchange had 38 ug of aluminium per gramme in bone and showed increased bone formation and resorption indicative of moderately severe

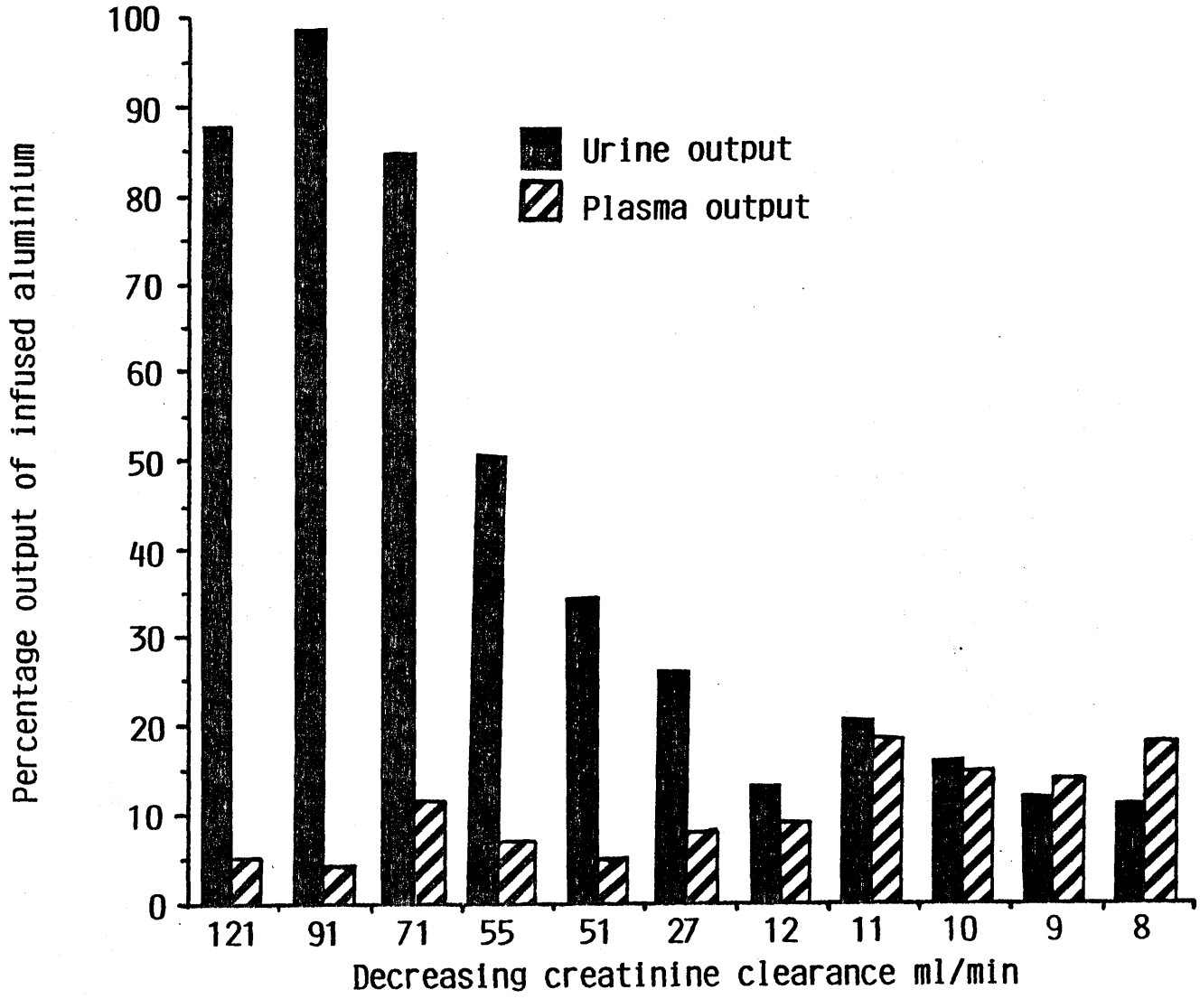


Figure 4.1 RENAL FUNCTION AND OUTPUT OF INFUSED ALUMINIUM IN URINE AND "PLASMA BAG"

Table 4.3 PLASMA ALUMINIUM CONCENTRATIONS BEFORE AND IMMEDIATELY AFTER PLASMA EXCHANGE

CASE	PLASMA ALUMINIUM BEFORE EXCHANGE (μmol/L)	PLASMA ALUMINIUM AFTER EXCHANGE (μmol/L)	% RETENTION OF ALUMINIUM
MC*	0.6	3.6	60.6
MC*	0.8	2.4	69.0
JM*	0.8	2.2	67.5
JM*	0.2	2.1	74.0
TS	2.1	2.7	70.6
SS	0.4	1.1	66.0
IM*	0.2	2.0	42.0
IM*	0.1	2.1	60.0
SW	0.7	1.3	3.6
HC	0.2	1.9	0
EA	0.1	1.7	6.5

* PATIENT STUDIED TWICE (INTERVAL ONE MONTH)

Table 4.4 - B O N E H I S T O M O R P H O M E T R Y

Bone Aluminium (Al)

Patient	AROS %	ALTS %	BONE Al ug/g	Type of Bone Disease
MC	0	0	38	2^{o} HPT with aluminium lines in calcified bones
JM	6.5	1.2	70	Normal bone turnover with aluminium along CF
TS	51.0	38.7	63	2^{o} HPT with aluminium along CF - mixed aluminium bone disease
IM	0	0	8	Normal bone turnover

2^{o} HPT = Secondary Hyperparathyroidism

Table 4.4 - B O N E H I S T O M O R P H O M E T R Y

Bone Formation and Resorption

Patient	TOS %	AOS %	CF %	MNL	TRS %	ARS %	NO PER MM²
MC	42.9	14.0	76.4	2	33.6	7.3	1.3
JM	11.2	1.1	80.9	2	2.3	0.5	0.13
TS	54.5	11.0	7.3	2	11.9	3.7	0.85
IM	5.4	0.6	61.9	1	9.5	0.1	0.14
Normal Value	<24%	<2.0	>60%	<4	<7.3%	<2.4%	<0.28

TOS = Total osteoid surface
AOS = Active osteoid surface
CF = Extent of calcification fronts
MNL = Maximum number of osteoid lamellae
TRS = Total resorption surface
ARS = Active resorption surface
NO = Number of osteoclasts

secondary hyperparathyroidism. Bone mineralization was normal and no aluminium was demonstrable histochemically at the calcified bone osteoid interface, although aluminium lines were present within calcified bone due to previous deposition. This patient complained of intermittent bone pain.

Patient JM after six months of weekly plasma exchange had 70ug of aluminium per gramme in bone and showed normal bone turnover, no impairment of mineralisation and minimal deposition of aluminium at the calcified bone osteoid interface.

Patient, TS after 84 months on plasma exchange had 63ug of aluminium per gramme in bone and showed extensive deposition of aluminium at the calcified-bone osteoid interface associated with a marked reduction in the extent of calcification fronts indicating defective mineralization. Osteoid seam thickness was not increased, therefore the appearances did not amount to frank osteomalacia. The changes of moderate secondary hyperparathyroidism were superimposed. This patient complained of intermittent bone pain.

Patient IM after 27 months on plasma exchange had 8ug of aluminium in bone, and a normal bone biopsy with no histologically stainable aluminium.

4.2.v DESFERRIOXAMINE MOBILIZATION TEST

The results obtained for the five patients are shown in the Table (4.5). The values for plasma aluminium before and after infusion are given and the difference (Δ plasma aluminium) at 48 hours calculated. Similarly the pre-infusion and post-infusion urinary aluminium excretions are given and the difference (Δ urine aluminium) is calculated.

The bone content of aluminium for MC,JM,TS and IM are shown alongside for comparison. Patients MC and JM for whom there was definite evidence of aluminium retention showed the greatest increases in both plasma aluminium concentration and in urinary aluminium excretion after desferrioxamine.

Patient TS who showed the greatest increment in plasma aluminium had been treated for the longest time (84 months). Urine samples for this patient were thought to be contaminated prior to analysis.

Patients IM and SW in whom there was less aluminium retention showed much smaller changes in plasma and urine aluminium after desferrioxamine.

Table 4.5 DESFERRIOXAMINE MOBILISATION TEST

	PLASMA ALUMINIUM (µmol/L)			URINE ALUMINIUM (umol/24H)			
CASE	BEFORE INFUSION	48 HOURS AFTER INFUSION	DIFFERENCE	BEFORE INFUSION	THREE DAYS AFTER INFUSION	DIFFERENCE	BONE ALUMINIUM (BEFORE INFUSION TEST) (ug/g)
MC	0.6	3.0	2.4	2.0	47.7	45.7	38.0
JM	1.2	3.6	2.4	8.4	72.6	64.2	70.0
TS	2.5	14.4	11.9	*	*	*	63.0
IM	0.1	0.1	0	2.4	17.9	15.5	8.0
SW	0.2	0.2	0	0.5	2.6	2.1	-

* URINE SAMPLES POSSIBLY CONTAMINATED BEFORE ANALYSIS

4.3. DISCUSSION

During experimental intravenous loading with aluminium, renal clearance is extremely efficient (152). It is not thought likely that endogenous secretion of aluminium into the gastrointestinal tract is an important route of elimination (150). Faecal aluminium output mainly reflects unabsorbed dietary aluminium. Therefore, measurement of faecal aluminium excretion was not attempted in the balance study. Of the eight patients who were being treated by plasma exchange for a variety of immunological disorders, four patients with severe renal impairment retained 60-70% of the aluminium infused during plasma exchange; while three patients with normal renal function retained very little aluminium (up to 6%). One patient with moderate renal impairment (creatinine clearance of 55 ml/min) retained 42 - 60% of the infused aluminium. A bone specimen obtained from this patient had a normal bone aluminium content and no stainable aluminium.

Transiliac bone biopsy specimens taken from three of the patients with high aluminium retention and poor renal function, showed an elevated bone aluminium content and histologically stainable aluminium. Although none of the patients had "classical" aluminium related osteomalacia and the pattern of aluminium staining differed amongst the three patients, these findings can be explained on the

basis of variations in bone turnover, the duration and frequency of plasma exchange and the total exposure of sites of bone formation to aluminium throughout the clinical history.

Patient MC had histological evidence of florid hyperparathyroidism since 1981 and again in the most recent bone biopsy. However in the interval following the administration of (1 -α - Hydroxy Cholecalciferol) she was biochemically euparathyroid for several years and it is considered that the aluminium lines within calcified-bone represent heavy deposition of the metal at the calcified bone interface during the euparathyroid phase. This patient did complain of intermittent bone pain.

The skeleton of patient JM, with normal bone turnover has not been exposed to aluminium for a sufficiently long period of time to cause major osteodystrophy, although there is a build up of aluminium in bone. This patient received weekly plasma exchanges compared to monthly exchanges for MC and TS.

Patient TS, with a milder hyperparathyroidism than patient MC, shows a "mixed" bone disease having a sufficiently low bone turnover to allow accumulation of aluminium at the calcified-bone osteoid interface, yet with sufficient turnover to offer a large osteoid surface for uptake of aluminium and produce defective mineralisation, but not

frank osteomalacia. This patient also complained of intermittent bone pain.

The plasma aluminium concentrations measured before and after exchange were not an accurate index of aluminium retention though there was a tendency for the patients with poor renal function to have the highest plasma aluminium concentrations immediately after exchange. The concentrations observed, however, did not exceed 3.7 umol of aluminium per litre which was suggested by Charhon et al.(153) as the level at which aluminium deposition in bone was probable in patients undergoing haemodialysis. These results show that aluminium retention can be present at plasma aluminium concentrations well below 3.7 umol of aluminium per litre.

Patient IM whose balance studies had shown some retention of aluminium is similar to the case reported by Milliner et al.(96) who showed that there was no bone biopsy evidence of aluminium toxicity in a patient with a normal serum creatinine, who had received a total of 83 plasma exchanges with aluminium contaminated albumin solutions. However the results clearly show that Milliner's conclusion "the amount of aluminium delivered by infusions of albumin is too small to present a real risk" is an overgeneralisation.

Demonstration of aluminium retention requires metabolic

balance studies and bone biopsy, and cannot be based upon observation of plasma aluminium concentrations alone.

Since none of the patients were being treated by haemodialysis and only one (patient MC) was receiving aluminium hydroxide as a phosphate binder (the two usual sources of aluminium in chronic renal failure), it is concluded that the aluminium accumulation in their bone has been due to the administration of aluminium contaminated albumin.

Although an aluminium induced osteomalacia is the best described effect of chronic aluminium toxicity, neurotoxicity is also well recognised and there is interest in the role of aluminium in the development of Alzheimer's disease and other degenerative conditions related to ageing.

Patients given infusions of contaminated albumin are receiving up to 10 times the amount of aluminium normally absorbed from the diet. The prolonged administration of large volumes of albumin (or other aluminium contaminated products) to patients with renal impairment will result in bone disease. There is also a possibility that these patients could be at risk of developing Alzheimer's disease in the long term.

CHAPTER 5.

STAGES OF MANUFACTURING PROCESS CONTRIBUTING TO CONTAMINATION OF ALBUMIN SOLUTIONS AND EFFICIENCY OF REVISED PROCEDURES TO REDUCE AND ELIMINATE THE CONTAMINATION

SOURCES OF CONTAMINATION

Introduction

Study Design

Results

REVISED PROCEDURES TO ELIMINATE CONTAMINATION

Introduction

Study Design

Results

DISCUSSION

5.1. SOURCES OF CONTAMINATION

5.1.i INTRODUCTION

In 1978, Elliott et al.(8) showed that albumin solutions were contaminated with aluminium. The Protein Fractionation Centre, Scottish National Blood Transfusion Centre, which was the manufacturer of albumin used in the patient study of Elliott et al. responded to this report and steps were taken to reduce the aluminium. The source of the aluminium contamination was thought to be sodium hydroxide solutions used during the manufacturing process.

When albumin solutions were tested eight years later (Chapter three) there was still significant aluminium contamination in most of the albumin solutions available for clinical use. Following an initial report on the level of aluminium and other trace metal contamination of albumin products (101), the various manufacturers of the products were advised of the levels of contamination. The unanimous response was that the levels of contamination reported were too low to be clinically significant. Following a second report,(154) (Chapter four) showing aluminium bone disease in patients with impaired renal function receiving albumin solutions, there were several responses from manufacturers of albumin solutions (155)(156)(157). In these reports there were clear indications of an attempt to remove or reduce metal

contamination in albumin solutions.

One of these manufacturers, the Protein Fractionation Centre, Scottish National Blood Transfusion Service initiated detailed and extensive investigations to identify the sources of metal ion contamination. When they had successfully identified the sources they then evaluated revised procedures intended to reduce and eliminate contamination.

Acknowledgement: The data in this section was kindly provided by Mr William McBey, Protein Fractionation Centre, Scottish National Blood Transfusion Service.

5.1.ii STUDY DESIGN

An outline of the manufacturing process of human albumin is shown in figure 5.1. Samples were taken at various intermediate stages (A-F) and analysed at the Scottish National Blood Transfusion Service by atomic absorption spectrometry using a Pye Unicam SP 192 spectrophotometer. The concentrations of aluminium, chromium, copper, manganese, nickel and zinc were determined in each sample.

Other possible sources of contamination which were studied included, sodium hydroxide solutions which are used for pH adjustment in the manufacturing process and caprylic acid solutions which prevent denaturation of albumin during pasteurisation. Stainless steel containers used throughout the manufacturing plant were also considered as a source of contamination and concentrations of the metal ions were determined in starting plasma before and after storage in a stainless steel container for a period of time similar to the time taken for the fractionation of albumin from plasma. The glass bottles used for storage of sodium hydroxide were also investigated by measuring the aluminium concentration in sodium hydroxide after storage times from 10 minutes to 24 hours.

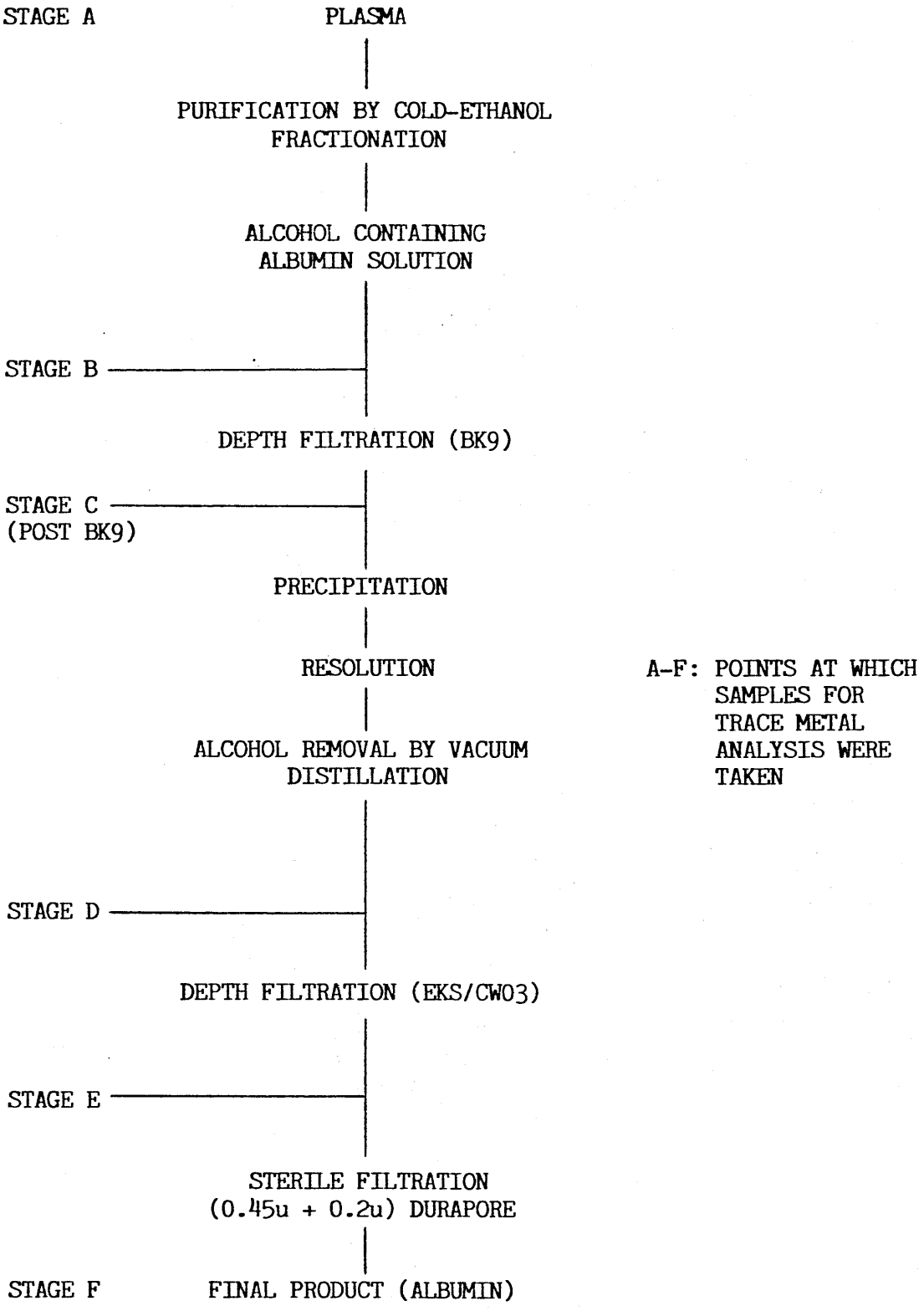


Figure 5.1 Flowsheet of plasma fractionation to albumin

5.1.iii RESULTS

Figure 5.2 shows that the concentrations of aluminium, nickel, chromium and manganese showed a steady increase throughout the process while copper concentrations were unaffected and zinc concentrations fell.

The depth filtration (Stage C) was found to be the principal cause of increased concentrations of trace metals, although the cartridge filters employed for sterile filtration Stage (F) resulted in a slight increase in the level of aluminium, chromium, nickel and copper.

Sodium hydroxide solutions were contaminated with aluminium and most of the metal had leached from glass storage containers (Table 5.1).

Caprylic acid contained significant concentrations of aluminium, ion and nickel as contaminants.

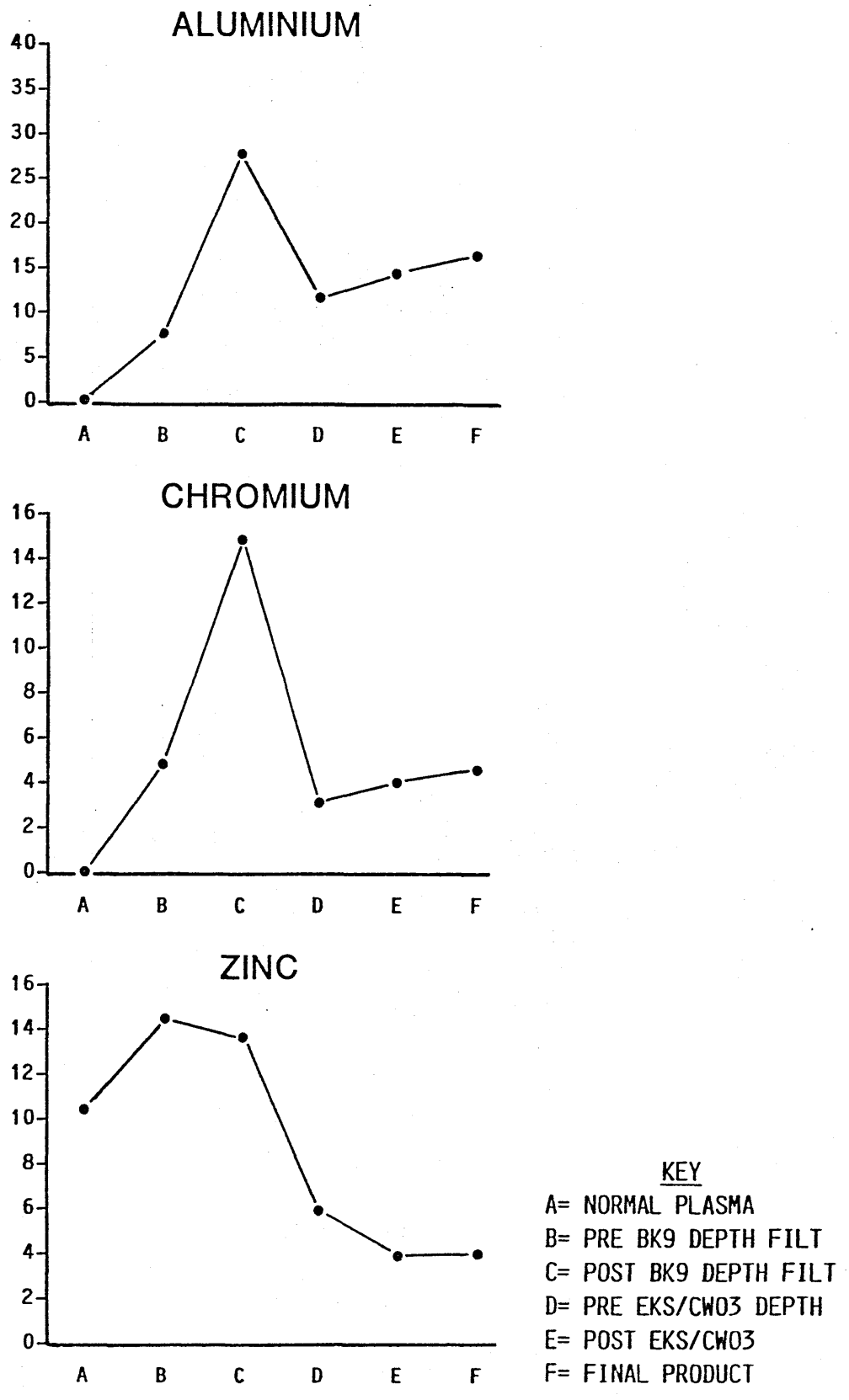


Figure 5.2 STAGES AT WHICH METAL CONTAMINATION OCCURS DURING ALBUMIN PRODUCTION.

METAL CONTAMINATION (ug/g)
V's
PROCESS STAGE FOR ALBUMIN

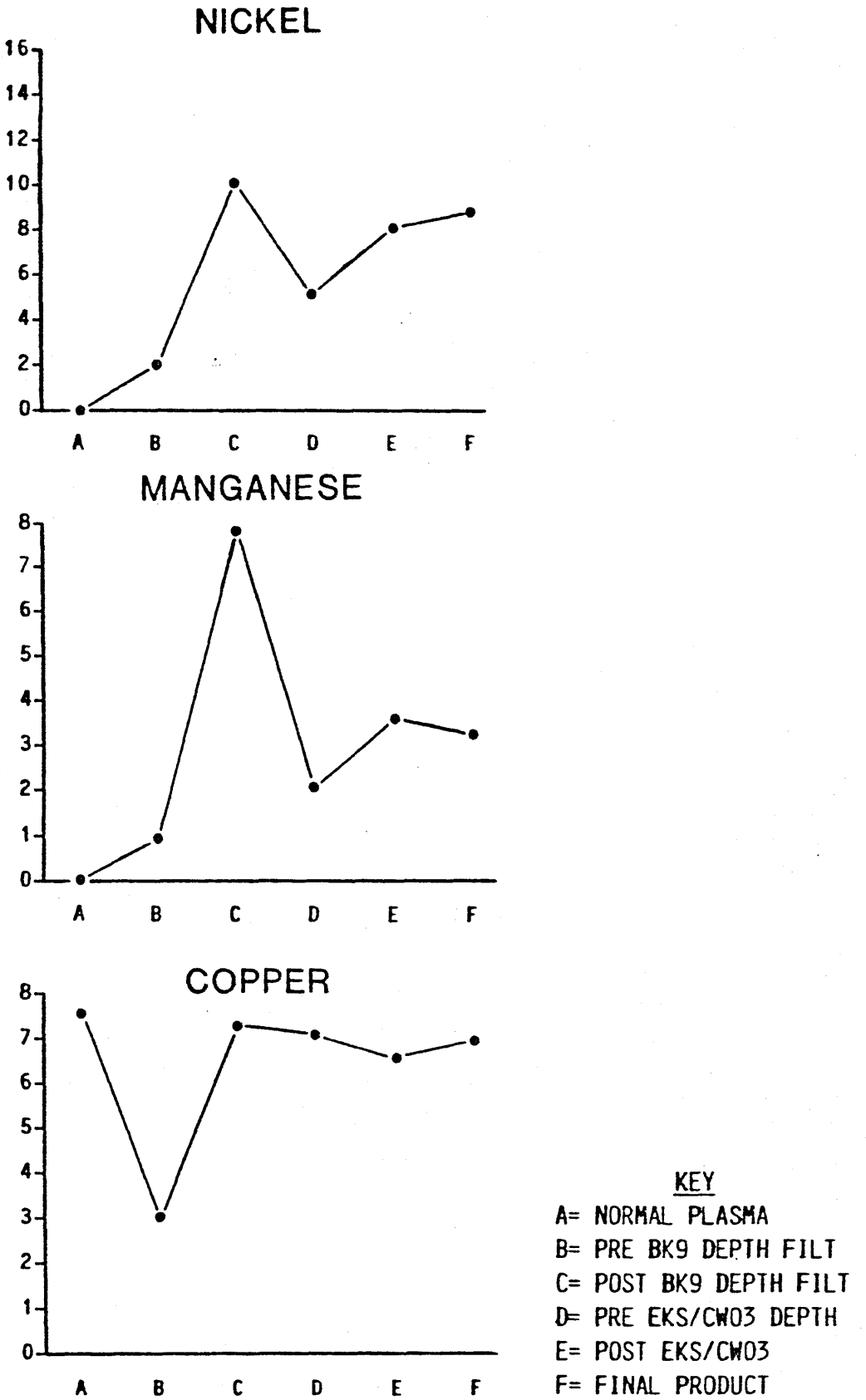


Figure 5.2 STAGES AT WHICH METAL CONTAMINATION OCCURS DURING ALBUMIN PRODUCTION.

Table 5.1 ALUMINIUM CONTENT OF NaOH*SOLUTIONS STORED IN VARIOUS CONTAINERS

TYPE OF CONTAINER	SOLUTION	STORAGE TIME	ALUMINIUM ppb
500 ML TYPE 1 DIN BOTTLE (GLASS)	5N NaOH	10 MINS 15 MINS 24 MINS	340 530 > 2000
100 ML TYPE II DIN BOTTLE (GLASS)	5N NaOH	10 MINS 15 MINS 24 MINS	320 470 > 2000
500 ML TYPE 1 DIN BOTTLE (GLASS)	0.05N NaOH	20 MINS 80 MINS 100 MINS 24 HOURS	15 180 345 2000
1 GALLON POLYCARBONATE (PLASTIC)	5N NaOH	20 MINS 90 MINS 24 HOURS 5 DAYS	15 15 15 15

* Na OH - Sodium Hydroxide

NOTE: The same relative amounts of sodium hydroxide was put into each container.

ppb = ug/l

5.2. REVISED PROCEDURES TO ELIMINATE CONTAMINATION

5.2.i INTRODUCTION

When the major sources of contamination of the manufacturing process were identified the Protein Fractionation Centre, Scottish National Blood Transfusion Service undertook a number of developments to further reduce contamination in albumin solutions and other blood products.

The filtration processes were modified so that the filters were flushed with a carefully selected wash solution (Citric Acid, pH3) before the passage of protein to reduce metal ion contamination.

The aluminium introduced into sodium hydroxide solutions during storage in glass containers was eliminated by replacing glass with plastic containers.

Caprylic acid was replaced by sodium caprylate which contained no significant amounts of either aluminium or nickel. Although the metal ion contamination could be reduced by these measures relatively high concentrations still remained in the albumin. The beneficial effect of ultrafiltration for the removal of both residual ethanol and metal ions then evaluated on a pilot scale.

Ultrafiltration is a process of selective molecular separation. It employs membranes capable of passing solutes and solvents of low molecular weight while retaining solutes that are above certain specified molecular dimensions. Diafiltration is defined as constant volume ultrafiltration.

5.2.ii STUDY DESIGN

Albumin solution (Stage F Figure 5.1) at pH 6.9 was diafiltered against five times its volume of 130 mmol sodium chloride using spiral wound cellulose membranes (Amicon low absorption YM series with 10,000 daltons molecular weight cut off). The resulting albumin solutions were pasteurised and the product re-analysed for metal ion contamination and evidence of protein denaturation and ethanol content.

5.2.iii RESULTS

The diafiltration process removed more than 90% of aluminium and manganese, 80% chromium, but only 25% nickel from the albumin solution (Table 5.2). The ethanol concentration fell exponentially with the volume of diafiltration solution (Figure 5.3).

The final pasteurised albumin solutions contained greatly reduced metal ion contamination when compared to the conventional product (Table 5.3). All quality control parameters were in the normal range.

Table 5.2 METAL ION REDUCTION DURING DIAFILTRATION

	METAL ION CONCENTRATION (ug/g PROTEIN)		
	ALBUMIN PASTE RESUSPENSION (PRE ULTRAFILTRATION)	POST ULTRAFILTRATION	% REMOVED BY ULTRAFILTRATION
ALUMINIUM	9.6 ± 5.7 (10)	0.45 ± 0.32 (10)	93.5 ± 5.4 (10)
CHROMIUM	2.3 ± 1.4 (5)	0.33 ± 0.09 (5)	81.4 ± 11.3 (5)
MANGANESE	2.1 ± 1.1 (6)	0.12 ± 0.24 (6)	96.4 ± 5.4 (6)
NICKEL	3.9 ± 1.7 (8)	3.0 ± 1.5 (8)	25.9 ± 21.0 (8)

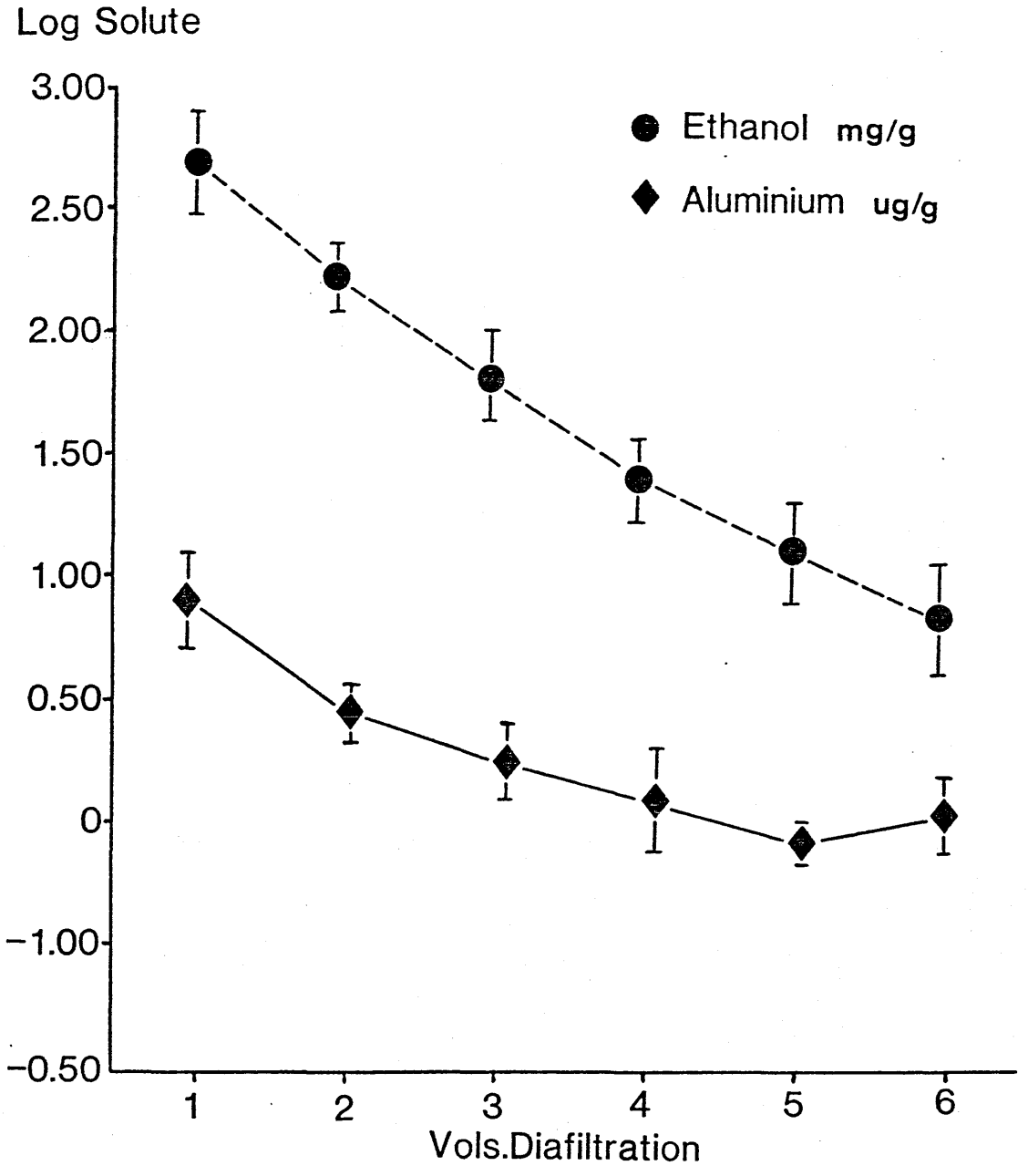


Figure 5.3 CHANGE IN ALUMINIUM AND ETHANOL CONCENTRATIONS DURING DIAFILTRATION.

Table 5.3 - METAL ION CONCENTRATION IN ALBUMIN (4.5%)

METHOD OF PREPARATION		STANDARD (CENTRI-THERM)	DIAFILTERED
	Units*		
ALUMINIUM	PPB	655 ± 171 (50)	29 ± 14 (12)
	umol/l	24.3	0.4
CHROMIUM	PPB	114 ± 15 (10)	21 ± 10 (9)
	umol/l	2.2	0.4
MANGANESE	PPB	206 ± 21 (10)	29 ± 15 (15)
	umol/l	3.7	0.5
NICKEL	PPB	231 ± 30 (9)	180 ± 69 (11)
	umol/l	3.9	3.1

* 1 PPB = 1 ug/l

RESULTS EXPRESSED AS MEAN ± SD WITH N IN BRACKETS

5.3. DISCUSSION

The detailed and extensive investigations undertaken by the Scottish National Blood Transfusion Service show that depth filters are the principal cause of increased concentrations of aluminium and trace metals presumably due to selective absorption and desorption taking place between the depth filter and the protein solution. These depth filters consist of a matrix of self-bonding fibres (cellulose) and diatomite, perlite or sand, and are therefore a rich source of aluminium and other trace metals.

The quantity of aluminium which can be leached from such filters into the product is reduced by extensive flushing with citric acid before use. The sources of contamination of sodium hydroxide solutions and caprylic acid can also be readily eliminated.

Despite these processing changes, some aluminium and trace metal contamination still occurs during the manufacturing process. This residual contamination is leached from the large stainless steel storage containers which are used in the manufacturing process. Most of the metals can be removed using an ultrafiltration procedure, but the small retention of metal ions is most likely the result of fractional binding to albumin with nickel being bound the tightest.

The results presented show that it is now possible to prepare albumin solutions with an aluminium content of less than 1 umol/l.

There have also been encouraging reports (97) from other manufacturers showing low concentrations of aluminium in their products following modifications to their manufacturing processes. Armour Pharmaceutical Company have carefully evaluated their albumin preparation methods and found that the source of contamination was the same as the Protein Fractionation Centre, Scottish National Blood Transfusion product. A new fractionation protocol which also included diafiltration was implemented by Armour. With this new process the aluminium concentration in 5% human albumin was reduced from 1108 $\pm$ 384 (41 $\pm$ 12.9) to 131 $\pm$ 20.1 ug/l (4.9 $\pm$ 0.7 umol/l) and the aluminium concentration in Armour 25% human albumin was reduced to 738 $\pm$ 81.6 mg/l (27.3 $\pm$ 3.1umol/l)(97). Inspite of these reductions the level of aluminium in these albumin solutions is four to twenty seven times higher than in normal plasma.

A recent report shows that the concentrations of aluminium in albumin solutions from most manufacturers remain unacceptably high (99). It is imperative that these manufacturers follow the example of the Protein Fractionation Centre, Scottish National Blood Transfusion Service and undertake detailed investigations of the

manufacturing process and institute the necessary modifications to produce albumin solutions which are not only free of aluminium but of other trace metals.

CHAPTER 6.

CITRATE AND ITS INTERACTION WITH METALS CONTAMINATING ALBUMIN SOLUTIONS

6.1. INTRODUCTION

There is a significant increase in aluminium intestinal absorption when citric acid is ingested orally at the same time (158). This occurs because the neutral complex of aluminium and citrate provides a means by which aluminium can pass through membranes. Similarly, the intraperitoneal administration of citric acid enhances urinary aluminium excretion (159). Citrate has been shown to be a very effective chelator of aluminium and other metals. Citric acid has been proposed as a possible therapeutic alternative to the use of desferrioxamine in the treatment of aluminium toxicity (160).

The ability of citrate to chelate calcium ions makes it a most efficient anticoagulant and it is added during the collection of whole blood before the plasma is separated and then fractionated into albumin and other blood products. These products may contain significant amounts of citrate, since it is not specifically removed during the production cycle.

It is likely that citrate will form complexes with aluminium and other trace metal ions present at various stages of the fractionation process. This role of citrate has not previously been considered by the manufacturers. In the scientific literature (161) over the last five years, there has been considerable interest in the

speciation of trace metals and their interaction with citrate.

Citric acid occurs as the tricarboxylate anion, citrate at pH 7.4 in blood and provides oxygen donor ligands which counter the 3+ charge on trivalent ions of the ferric, chromium or aluminium type. The carboxylate groups in citrate are positioned to make stronger binding possible with these trivalent ions compared with other common divalent ions such as calcium, magnesium, ferrous copper, zinc and manganese. The stability constants give an indication of the relative binding strengths of the citrate ligand with these ions, many of which are present as contaminants in blood products (161).

The neutral complexes formed between citrate, aluminium and other trace metals would be expected to pass through cell membranes of the renal glomeruli. When infused in albumin metal-citrate should be rapidly excreted in the urine. The studies in Chapter four confirm that this statement is true for aluminium. Although the interactions are complex it is possible that ions such as ferric, chromium and aluminium with higher binding constants to citrate would be excreted more efficiently than other ions such as manganese with lower stability constants.

The contamination of albumin with citrate, aluminium and

other trace metals, and its infusion into patients with normal renal function provides a unique opportunity to study the effect of citrate on the speciation of these metals and its role in reducing toxicity by enhancing renal elimination.

Several collaborative investigations were undertaken with the following objectives.

1. To assess citrate concentrations in albumin and at various stages of the manufacturing process.

2. Gel filtration studies to investigate the speciation of metal ions aluminium, chromium, manganese, selenium, nickel and the proportion of these ions associated with citrate.

3. The effect of ultrafiltration on the concentrations of citrate, aluminium, chromium, manganese, iron and citrate in albumin.

4. Comparison of balance studies for aluminium, chromium, manganese and iron using ultrafiltered albumin with similar studies using contaminated albumin.

6.2. CITRATE AND ALUMINIUM CONCENTRATIONS AT VARIOUS STAGES OF PRODUCTION CYCLE OF ALBUMIN INCLUDING THE NEW ULTRAFILTRATION STEP

6.2.i STUDY DESIGN

Samples were taken from two continuous runs of the albumin (4.3%) production cycle of the Protein Fractionation Centre, Scottish National Blood Transfusion Centre.

Aluminium

Aluminium concentrations were determined by electrothermal atomic absorption spectroscopy.

Citrate

The citric acid content of the samples was determined using a UV-method (Boehringer-Mannheim GMBH, Biochemica 1986), based on the following:

a) Citrate $\xrightarrow{\text{lyase}}$ oxaloacetate + acetate.

b) Oxaloacetate + NADH + H^+ $\xrightarrow{\text{Malate dehydrogenase}}$ L-Malate + NAD^+

c) Pyruvate + NADH + H^+ $\xrightarrow{\text{Lactate dehydrogenase}}$ L-Lactate + NAD^+

The amount of NADH oxidised in reactions b and c is stoichiometric with the amount of citrate. NADH is determined by means of its absorbance at 340nM.

Reagents were obtained in a test kit (Citric acid - UV method, Biochemica) and determinations carried out using a Cobas Mira spectrophotometer.

6.2.ii RESULTS

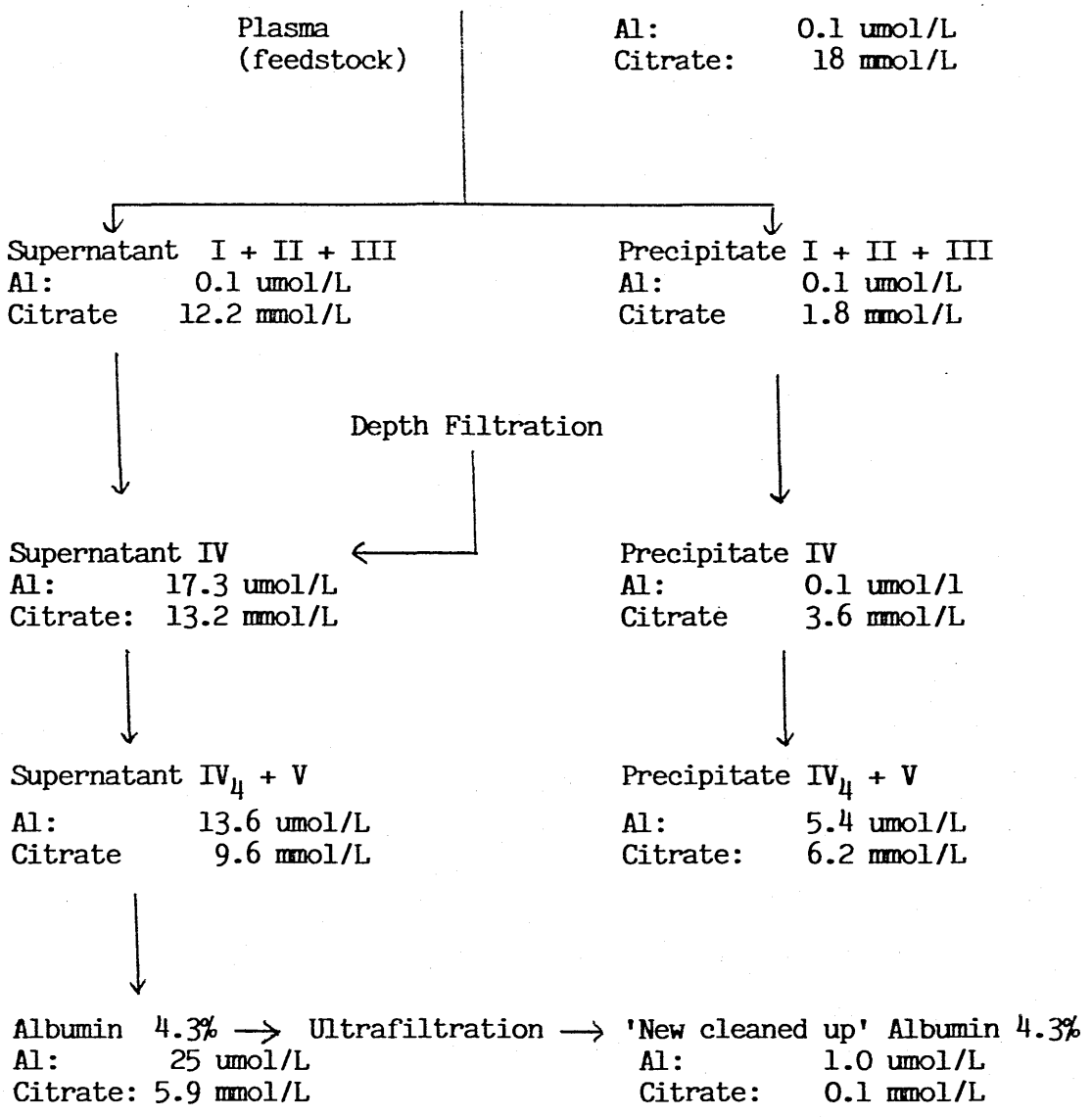
Citrate concentrations remain high throughout the fractionation process and can therefore chelate aluminium and metal ions which are present during the production cycle (Figure 6.1).

This study confirms the results of Chapter five which showed that most of the contaminating aluminium was added during the depth filtration stage and following the ultrafiltration step there are low concentrations of contaminant aluminium and citrate in albumin solutions.

Acknowledgement: The data in this section was kindly made available by Mr William McBey, Protein Fractionation Centre, Scottish National Blood Transfusion Service.

Figure 6.1

CITRATE AND ALUMINIUM LEVELS THROUGHOUT THE FRACTIONATION PROCESS (CSVM)



6.3. THE EFFECT OF ULTRAFILTRATION ON THE CONCENTRATIONS OF ALUMINIUM, CHROMIUM, MANGANESE, IRON AND CITRATE IN ALBUMIN SOLUTIONS

6.3.i STUDY DESIGN

Aluminium, chromium, manganese and iron concentrations were determined by inductively coupled mass plasma spectrometry. Citrate concentrations were determined as previously described.

Batches of albumin (4.3%) produced by the Edinburgh Protein Fractionation Centre, Scottish National Blood Transfusion Service were selected at random from supplies of albumin available for clinical use in June 1989 in the Blood Products Laboratory of the Royal Infirmary, Glasgow. In addition, samples of albumin (4.3%) (ultrafiltered) were supplied by the Edinburgh Protein Fractionation Centre Scottish National Blood Transfusion Centre. For comparison a batch of albumin (331050720) manufactured before the introduction of the new ultrafiltration process was also tested.

6.3.ii RESULTS

In the 'new' albumin solutions, citrate concentrations are now normal (Table 6.1). Aluminium has also been reduced to plasma reference concentrations of around 1 umol/l or less and the problem of aluminium toxicity secondary to albumin should now be eliminated.

There have been marked reductions in chromium, manganese and iron concentrations and their concentrations are marginally elevated above the reference plasma range. There is some variation between batches and frequent monitoring will be required to ensure that the concentrations remain low.

Table 6.1 ALUMINIUM, CHROMIUM, MANGANESE, IRON AND CITRATE LEVELS IN ALBUMIN (4.3%) SOLUTIONS

	ALBUMIN (4.3%)	PS8 (ULTRAFILTERED)	ALBUMIN (4.3%) (ULTRAFILTERED)	"NORMAL PLASMA" REFEREMCE RANGE
Batch Number	331050720	Experimental 1	331182710	
Date of Issue	April 1986	February 1988	July 1989	
Al umol/L	18.3	0.7	1.0	<0.5
Cr nmol/L	2,500	200	504	<5.0
Mn nmol/L	6,000	200	174	7-27
Fe umol/L	175	25	41	8-30
Citrate mmol/L	5.9	0.1	0.1	<0.1

6.4. GEL FILTRATION STUDIES ON ALBUMIN TO DETERMINE THE SPECIATION OF ALUMINIUM, CHROMIUM, MANGANESE, IRON AND CITRATE IN ALBUMIN SOLUTIONS

6.4.i STUDY DESIGN

A disposable "PD10" column (Sephadex G25) was loaded with 2.5ml of 5% albumin. A Volume of 3.5ml of phosphate buffer was then added and the effluent collected for analysis. The material passing through the column is considered to be the protein-bound fraction. The fraction remaining on the column has entered the gel and is of low molecular weight (10,000 Dalton or less).

Aluminium, chromium, manganese, iron and nickel concentrations were determined using inductively coupled plasma mass spectrometry. Citrate concentrations were determined as previously described. The percentage of the initial metal and citrate present in albumin as a low molecular complex is calculated.

6.4.ii RESULTS

No data is available for chromium due to analytical problems.

The albumin solutions contained 5.7mmol/l citrate (Table 6.2)which is similar to the citrate level obtained by the Protein Fractionation Centre, Scottish National Blood Transfusion Service for a different batch of 4.3% albumin (Figure 6.1).

Most of the aluminium and chromium present in albumin is bound to citrate in a low molecular weight fraction (10,000 Daltons) and is therefore ultrafilterable. This is confirmed by the close correspondence of these results to the ultrafilterable fractions reported by the Protein Fractionation Centre Scottish National Blood Transfusion Service.

Nickel is highly protein-bound and therefore has a low ultrafilterable fraction. Significant amounts of this metal will remain as a contaminant in albumin solutions.

The calculations show that about 50% of iron should be present as citrate complexes. However these results have to take into account the competitive binding of iron with transferrin which is present in varying amounts in different batches of albumin.

Table 6.2

CITRATE AND METAL CONCENTRATIONS IN ALBUMIN BEFORE AND AFTER FRACTIONATION THROUGH SEPHADEX COLUMN.

METAL AND CITRATE	BEFORE FRACTIONATION	AFTER FRACTIONATION		Low mol. wt. Fraction as a % of total	% * REMOVED BY ULTRAFILTRATION (Taken from Table 5.2)
		PROTEIN BOUND	LOW MOLECULAR WEIGHT		
Al/umol	32	6	26	81.3	93.5
Mn/umol	3.5	0.8	2.7	84	96.4
Fe/umol	114	61	53	46.5	-
Ni/umol	7.5	6.3	1.2	16	25.9
Citrate/mmol/l	5.7	0.360	5.344	94	

* (Edinburgh Protein Fractionation Centre
((Scottish National Blood Transfusion Service

6.5. COMPARISON OF BALANCE STUDIES FOR ALUMINIUM CHROMIUM, MANGANESE AND IRON USING ULTRAFILTERED ALBUMIN WITH SIMILAR STUDIES USING CONTAMINATED ALBUMIN

6.5.i GENERAL CONSIDERATIONS

Since most of the aluminium and other metals (except nickel) and citrate have been removed by ultrafiltration from albumin solutions, it can be assumed that there should be very little urinary excretion of metals in patients with good renal function.

6.5.ii STUDY DESIGN

To test this hypothesis, balance studies for aluminium, chromium, manganese and iron were carried out on a patient (creatinine clearance 52 ml/m) receiving plasma exchange with the 'new' ultrafiltered albumin solution (Table 6.3). The protocol for plasma exchange, and balance studies were the same as for patients studied in chapter four. One of the previously studied patients (IM) and with a similar level of renal function (creatinine clearance 55mls/min) was used as a control (Table 4.1). This patient had received the 'old' citrate and metal contaminated albumin and the urinary excretion of aluminium, chromium, manganese and iron were measured.

Table 6.3 CLINICAL DETAILS OF PATIENTS (METABOLIC BALANCE FOR Al.Cr. Mn.Fe)

CASE	SEX AND AGE (YEARS)	DIAGNOSIS	TIME ON PLASMA EXCHANGE	TOTAL NO. OF PLASMA EXCHANGES	TOTAL VOL. OF ALBUMIN RECEIVED (L)	CREATININE CLEARANCE (ml/min)
CR	M 45	GUILLAIN-BARRE SYNDROME	4 MONTHS	12	36	52
IM	M 52	WALDENSTROM'S MACROGLOBULINAEMIA	2 YEARS 3 MONTHS	60	144	55

6.5.iii RESULTS

The load of aluminium infused in the 'new' ultrafiltered albumin solution is now negligible and is in fact less than the daily basal excretion of aluminium absorbed from natural sources (Table 6.4). There is no change in plasma concentrations or urinary excretion post-plasma exchange and most of the aluminium infused is removed in the exchange plasma (Table 6.5). The results show that the concentrations of aluminium in the ultrafiltered albumin solutions no longer pose a threat of toxicity in patients with impaired renal function.

The small amounts of chromium, manganese and iron infused in the 'new' ultrafiltered albumin do not cause any observed change in plasma concentrations (Table 6.5). The exchange fluid is now the main route of elimination of those metals during plasma exchange since there is no net urinary excretion of these metals (Table 6.4).

These results contrast sharply with the high concentrations of aluminium, chromium, manganese and iron observed in the patient with a similar level of renal function and who was plasma exchanged with the contaminated albumin (Figure 6.2, Figure 6.3).

These results confirm the original hypothesis and suggests that citrate has an important role in the renal

Table 6.4 METABOLIC BALANCE STUDIES: ALUMINIUM, CHROMIUM, MANGANESE, IRON

Patient: CR

METAL	METAL INFUSED IN ALBUMIN	METAL EXCRETED IN URINE OVER TWO DAYS	METAL REMOVED DURING PLASMA EXCHANGE
ALUMINIUM (umol/L)	2.0	0	1.4
CHROMIUM (nmol/L)	1411	7.5	1414
MANGANESE (nmol/L)	487	0	73
IRON (nmol/L)	114	0	29.5

Table 6.5 - PLASMA METAL CONCENTRATIONS BEFORE AND IMMEDIATELY AFTER PLASMA EXCHANGE

Plasma Levels

	Al umol/l	Cr nmol/l	Mn nmol/l	Fe umol/l	Citrate (mmol/l)
Pre-exchange:	0.3	5.2	27.6	9	0.1
1st day post:	0.5	32.7	18.9	20	0.1
2nd day post:	0.4	21.7	26.8	8	0.1

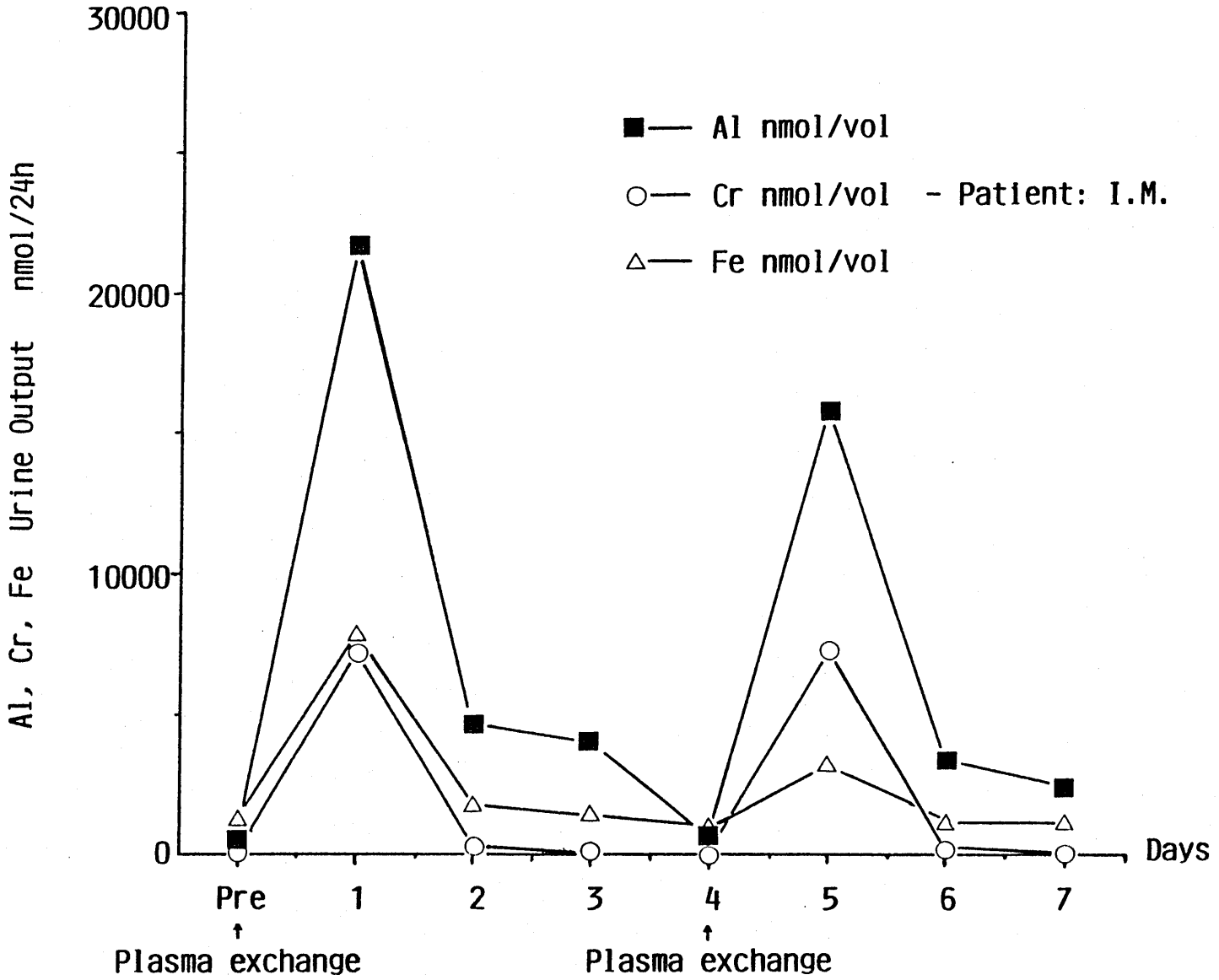


Figure 6.2 URINE OUTPUT OF ALUMINIUM (Al), CHROMIUM (Cr) AND IRON (Fe) AFTER PLASMA EXCHANGE

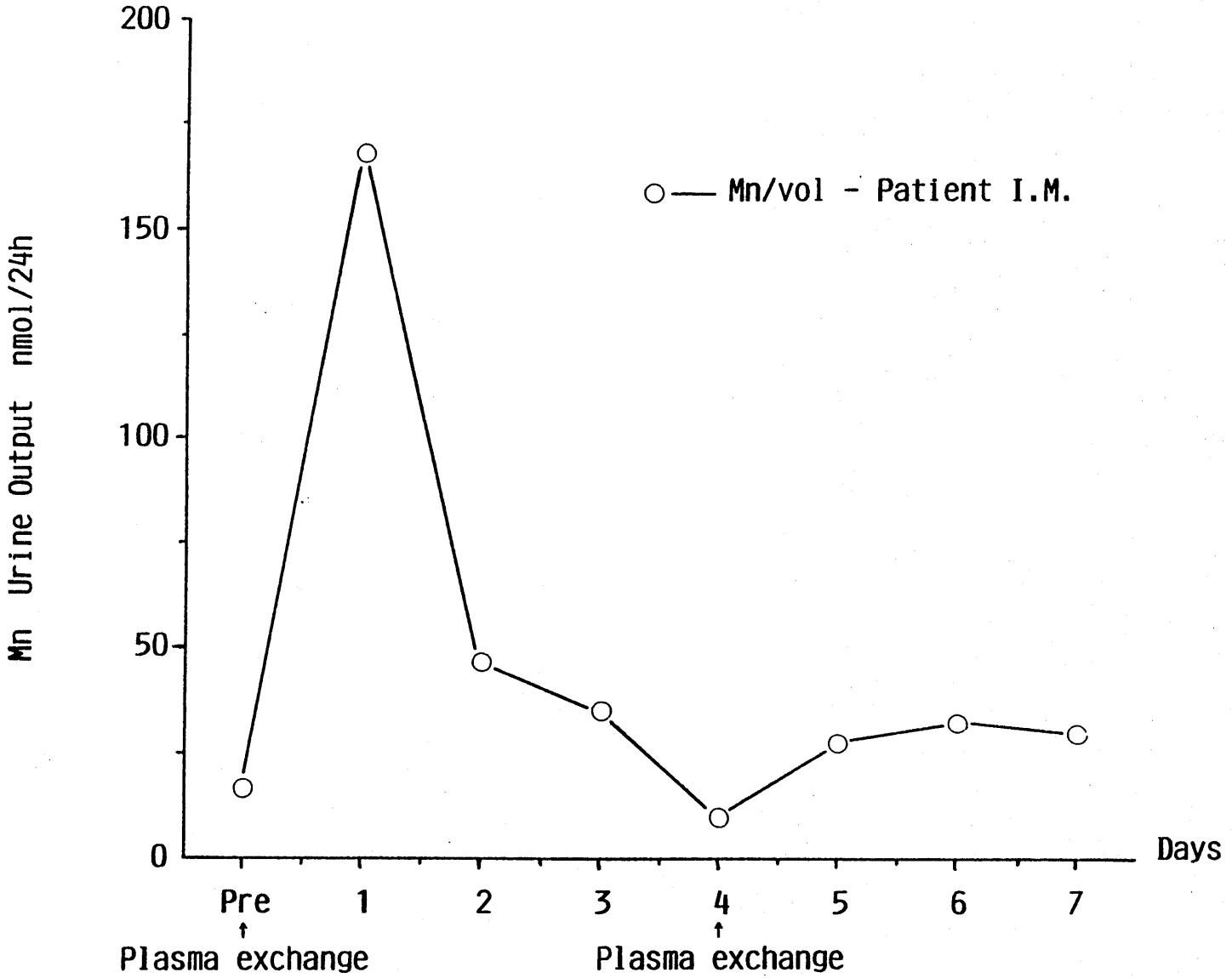


Figure 6.3 URINE OUTPUT OF MANGANESE (Mn) AFTER PLASMA EXCHANGE

elimination of these metal ions as a result of the formation of low molecular weight citrate-metal complexes.

6.6. DISCUSSION

The gel filtration data and the metabolic balance studies provide experimental and clinical evidence for the important role of citrate in the speciation of metal ions in albumin solutions. It is therefore very likely that citrate prevents metal retention and toxicity in those patients with normal renal function by promoting a most efficient renal excretion of aluminium and other metals with which it forms neutral low molecular weight complexes.

Further evidence for this important role of citrate is seen when the incidence of bone disease in patients with normal renal function receiving aluminium contaminated total parenteral nutrition (casein hydrolysate) is compared with similar patients receiving aluminium contaminated albumin.

The total parenteral nutrition caused aluminium bone disease in patients with normal renal function (88) while patients with good renal function who were injected with large amounts of aluminium in albumin during long term plasma exchange showed no evidence of bone disease (Chapter four) (96)(99). The percentage urinary excretion of an aluminium load in patients receiving total parenteral nutrition varied from 5 to 20% compared to almost 100% in patients receiving albumin (Chapter four).

An important difference between the two intravenous sources of aluminium contamination is that the total parenteral nutrition contained no citrate whereas albumin solutions contain ten to fifteen times the normal plasma citrate concentrations.

The 'new' ultrafiltered albumin solutions show normal concentrations of aluminium and marginally elevated values for chromium, manganese and iron with negligible retention. These ultrafiltered albumin solutions should be safe for injection in patients with impaired renal function.

CHAPTER 7

FINAL DISCUSSION AND CONCLUSION

CONCLUSION

These results provide evidence for a previously unrecognised toxic effect of treatment with albumin solutions.

The albumin solutions analysed contained up to forty times the aluminium normally present in fresh frozen plasma, the starting material for its manufacture. Other blood products including coagulation factors such as factor VIII, Prothrombin Complex, as well as immunoglobulin preparations had similar concentrations of aluminium contamination. There was also a widely varying trace metal contamination of albumin and blood products which occurred during the manufacturing process.

A recent report,(99) shows that the concentrations of aluminium in albumin solutions from most manufacturers remain unacceptably high.

As a direct result of the information on aluminium toxicity in this thesis, two manufacturers Merieux (157) and the Protein Fractionation Centre, Scottish National Blood Transfusion Service, Edinburgh,(154) have introduced stages in the manufacturing process to remove the aluminium and trace metals which contaminate their albumin products.

The Protein Fractionation Centre, Scottish National Blood Transfusion Service have carried out detailed and extensive investigations of their manufacturing process. They have identified the sources of contamination which include depth filters, sodium hydroxide solutions used for pH adjustment, stainless steel processing equipment and stabilisers such as caprylic acid. They have successfully introduced an ultrafiltration step and recent analysis shows that their albumin solutions are the 'cleanest' now available commercially. The results of their investigations will be of value to manufacturers who use a similar process and should encourage other manufacturers to follow their example.

It is understandable that until recently manufacturers have been reluctant to accept that albumin solutions are a potential cause of metal toxicity. There is now considerable evidence in the literature for aluminium as a toxic agent. However, the biological activity and clinical importance of marginal depletions or accumulation of other trace elements remains uncertain. At present unequivocal evidence is not available to show that these contaminating metals pose health risks.

The toxicity of aluminium is clearly recognised and this cannot be ignored by the manufacturers of contaminated albumin and clinicians who treat their patients with these products.

In patients with chronic renal failure, aluminium has been implicated in the pathogenesis of Vitamin D resistant osteomalacia, dialysis encephalopathy and microcytic anaemia.(1)

The administration of albumin to patients with renal insufficiency is of particular importance since the kidney is the main route for aluminium elimination and 60 to 70% of an aluminium load may be retained, resulting in bone disease.

The pattern of aluminium bone disease is dependent on parathyroid gland activity as well as variations in the duration and frequency of aluminium loads administered in albumin solutions. Extensive aluminium bone deposition is seen in euparathyroid patients receiving weekly plasma exchanges with albumin.

Intravenous desferrioxamine infusions increase the urinary output of aluminium and may be a useful therapeutic procedure in patients with evidence of aluminium retention.

In individuals with normal renal function, who receive aluminium contaminated albumin, the kidneys excrete most of the aluminium load and significant bone pathology is not seen. In contrast, aluminium bone disease occurs in patients with normal renal function, who receive total

parenteral nutrition containing casein hydrolysate with similar concentrations of aluminium as albumin (87). The serum binding and urinary excretion patterns of aluminium and trace metals are different in these two groups of patients. In the total parenteral nutrition group, aluminium binds strongly to plasma proteins with a low excretion of aluminium and resultant bone disease. Whereas in the albumin group almost all of the contaminating metals are excreted in the urine.

Citrate concentrations in albumin are ten to fifteen times greater than in normal plasma. It is an efficient chelator of aluminium and other metal ions in plasma and it is likely that the citrate contamination of albumin is protecting patients with normal renal function by enhancing metal excretion and reducing toxicity.

In patients with renal impairment, aluminium citrate accumulates and this is a potent bone poison which accelerates the development of osteomalacia (120).

Although an aluminium induced osteomalacia is a well established effect of chronic aluminium toxicity, neurotoxicity is also recognised and there is interest in the role of aluminium in the development of Alzheimer's disease and other degenerative conditions related to ageing.

In Martyn's report (3) the risk of Alzheimer's disease was one and a half times higher in districts where the mean concentration of aluminium in water exceeded 4 umol/l. The absorption of aluminium from the gastrointestinal tract is very small and less than 1% of the aluminium present in water would be expected to enter the blood stream. The concentrations in water are very low compared to the 40 umol/l of parenteral aluminium infused with each litre of albumin solution.

There is considerable debate over the role of aluminium as the primary cause of Alzheimer's disease, but nevertheless if the association between Alzheimer's disease and aluminium is true then patients who retain aluminium while receiving treatment with albumin could be at risk of developing this disease.

The current European Economic Commission Directive limits the aluminium concentration of drinking water to 7.4 umol/l (200ug/l). In view of the recently recognised risk of aluminium toxicity from albumin solutions a tentative reference value of 7.4 umol/l was agreed at a recent European Pharmacopoeia Commission meeting.

There is a great contrast in the attitudes of authorities like the European Pharmacopoeia Commission to metal toxicity in relation to aluminium and other trace metals when compared with the present ideas in relation to lead,

mercury and arsenic. If these heavy metals were present as contaminants in albumin solutions then manufacturers would have been under greater pressure to eliminate this contamination. The major emphasis in safety in the production of blood products is rightfully on microbiological and viral contamination. However, modern analytical techniques such as atomic absorption spectroscopy and inductively coupled plasma mass spectrometry can show up low concentrations of metal contamination which have not been formerly recognised.

It is probably fortuitous that the increase in aluminium in albumin is accompanied by an increase in citrate, otherwise this product would have been manifestly and immediately toxic in patients with normal renal function.

On the basis of the present evidence if manufacturers are to produce an ideal albumin solution for clinical use then apart from maintaining bacteriological and viral sterility they must aim at restoring the metal content of these solutions to concentrations as close as possible to normal plasma. They should aim to remove low molecular weight contaminants such as alcohol, citrate and citrate complex metals as well as highly protein bound metal contaminants such as nickel.

APPENDIX I.

ALUMINIUM (A1) METABOLIC BALANCE STUDY

Example of calculation

Patient:	I.M.
Date of Plasma Exchange	13th April
Total Volume of Plasma Exchanged	2.8 Litres
Batch No. of Albumin 4.3%	331050720
Aluminium Level (331050720)	19.7 umol/l
Total Aluminium Infused	55.2 umol

ALUMINIUM OUTPUT

Exchange Fluid

Date	Volume /Litres	Al Level (umol/l)	Total Al Level (umol)
13.4	Bag 1: 1.6	1.8	2.9
	2: 0.8	1.7	1.4

Aluminium Output = 4.3 umol.

URINE

Before Plasma Exchange

Basal Urinary Aluminium Level

24 hr Urine Volume x Urine Al Level (umol/l)

1.85 x 0.4

= 0.7 umol

After Plasma Exchange

Date	Volume (L)	Al/Conc. (umol/l)	Al Output (umol)
13-14/4	1.60	13.5	21.6
14-15/4	1.95	2.4	4.7
15-16/4	2.0	2.0	4.0

Aluminium Output = 30.3 umol

Urinary Aluminium (Aluminium output - 3 day Basal Urine Aluminium Conc.)

= 28.1 umol

Total Aluminium Output (Urine and Exchange Fluid)

= 32.3 umol

% Aluminium Recovery =

Total Al Output ÷ Al infused in Albumin x 100

= 58%

APPENDIX II

DESFERRIOXAMINE (DFO) MOBILIZATION TEST

Example of Calculations

Patient: M.C.

Plasma

Plasma Aluminium Concentrations (umol/l)

Pre DFO - 0.6

Post DFO

24 hrs - 3.2

48 hrs - 3.0

72 hrs - 1.8

Increase in Plasma Aluminium Level (umol/l)

= Post DFO - Pre DFO

24 hrs - 2.6

48 hrs - 2.4

72 hrs - 1.2

URINE

Urine Volume(L) (24 hours)	Urinary Al (umol/l)	Urinary Al Content (umol) (Urine Vol x Urinary Al)
Pre DFO		
1.2	1.1	1.3(basal Al)
Post DFO		
1st 24 hr		
1.1 L	19.2	21.1
2nd 24 hr		
1.4 L	14.6	20.4
3rd 24 hr		
1.4 L	8.7	12.2

Net Urinary Aluminium Level (umol) =

Urinary Al - Basal Al Excretion

24 Hour Period Post DFO	
1st	19.8
2nd	19.1
3rd	10.9

Total Urinary Aluminium Excretion Post DFO Infusion

= 49.8 umol/l

APPENDIX III

PLASMA PROTEIN FRACTIONATION AND PREPARATION OF BLOOD PRODUCTS

Introduction

Almost 40 years after the pioneering work of E.J. Cohn and his associates, most of the plasma processing throughout the world is still fractionated following the basic alcohol method (162). There have been modifications,(163) but the aim of most of these changes has been the economical production and good yield of the few proteins that are used in large quantities in clinical practice. The fractions produced for clinical use are albumin (5% or 20%), immunoglobulins, Factor VIII concentrates, concentrates containing Factor IX in association with other coagulation factors and antithrombin III concentrates.

Ethanol: Cohn Fractionation

Ethanol has been used now for several decades in plasma fractionation and it causes precipitation of proteins mainly because it significantly lowers the dielectric constant of the aqueous solution. The following properties of ethanol make it ideal for organic solvent precipitation:

1. Miscibility with water.
2. Melting point depression to - 22 °C. at a concentration of 32% (w/w).
3. No generation of explosive gas mixtures under normal ambient and working conditions.
4. Low molecular weight of only 46 daltons.
5. Highly volatile
6. Chemically relatively inert
7. Low toxicity
8. Inexpensive and easily available

The advantage of ethanol which is of paramount importance in a fractionation process is its inhibition of bacterial growth and thus of pyrogen formation. This inhibition also results indirectly from the low temperatures needed when working with ethanolic protein solutions.

The preparation of pure proteins (Table A III.1) from plasma is facilitated by the influence of several factors on the precipitation action of alcohol. These factors are pH, ionic strength, temperature and protein concentration, i.e. the "five parameter system". Separations are carried out in two different ways:

Conditions are chosen to maximise solubility of the desired protein and minimise solubility of all others. The desired protein stays in solution while all others precipitate, or the converse conditions are chosen where

Table A III.1 DISTRIBUTION OF MAJOR PLASMA PROTEINS IN COHN FRACTIONS

FRACTION						
I	II	III	IV-I	IV-4	V	V-Supernatant
Factor VIII	IgG	IgM	Ceruloplasmin	Trans-ferrin	Albumin	$Alpha_1$ acid glycoprotein
Fibrin ogen		IgA	Antithrombin III	Hapto globin		
		$Alpha_2$ macro globulin	$Alpha_1$ antitrypsin			
		Prothrombin complex				
		plasminogen				

the desired protein will then be selectively precipitated.

Plasma fractionation is carried out in large tanks or jacketed vessels with agitation. Adjustment to pH, ionic strength and protein concentration is made in these vessels either by direct titration or by calculation following titration of samples from the bulk pool. When precipitation is complete and the mixture has been allowed to equilibrate fully, it is then passed to a filter or a temperature controlled centrifuge for phase separation. The process is a semi - continuous flowing stream which is conditioned sequentially and separated without stopping.

At donor centres whole blood is collected in donation packs which contain Citrate - Dextrose - Phosphate Adenine as the anticoagulant. Plasma is separated from the cells into single donation plasma packs, frozen and transported to the fractionation centre where it is stored frozen at - 40 °C until required.

Liquid-solid separations are critical in all plasma fractionation procedures in order to harvest desired products and remove contaminants such as lipids, denatured proteins and micro-organisms. Filtration and centrifugation are the two methods generally used. Filtration is used after centrifugation steps as a polishing operation for the removal of small amounts of solids in order to achieve clarification, purification,

stabilization and final sterilization.(160) Depth filters are most often used as prefilters upstream of surface filters that usually provide final clarification and sterilization.

The objective of prefiltration is to increase the filterability of the fractions, to and though the final filter, so that a satisfactory through-put and rate can be achieved.

A surface or screen filter is a geometrically regular porous matrix that traps particles at its surface by a sieving mechanism. The surface filter is not designed for high dirt-loading capacity and its rapid plugging is typical. For this reason, surface filters normally used for sterilization, stabilization or clarification are usually preceded by depth filters.

A depth filter has a random and tortuous porosity and is capable of retaining particles throughout its matrix rather than solely on the surface.

Preparation of Albumin and Other Blood Products

Prior to fractionation the frozen plasma is pooled by passing the frozen blocks through an enclosed ice-crushing plant and then to a large pooling vessel. Cryoprecipitation is the first step (Figure A III.1) and results from the formation of regions of high concentrations of solutes such as proteins and salts in the inter-crystalline spaces of the ice lattice during freezing. Frozen plasma is allowed to thaw slowly to 4 C and the cryoprecipitate collected in continuous flow centrifuges. Intermediate factor VIII concentrates are then produced by extraction of the cryoprecipitate with buffer, and this is followed by an absorption with aluminium hydroxide to remove prothrombin and other contaminants. The intermediate purity factor VIII so produced undergoes sterile filtration for clarification of the product and lyophilization for solvent removal and freeze drying prior to heat treatment to inactivate viruses including HIV.

Ethanol fractionation of the supernatant following cryoprecipitate removal results in five fractions, I, II and III, IV-4 and V, as shown in figures (Figure A III.2).

Fraction I was used for preparing fibrinogen, which is no longer a licensed product due to its high risk of hepatitis and limited clinical limitations.

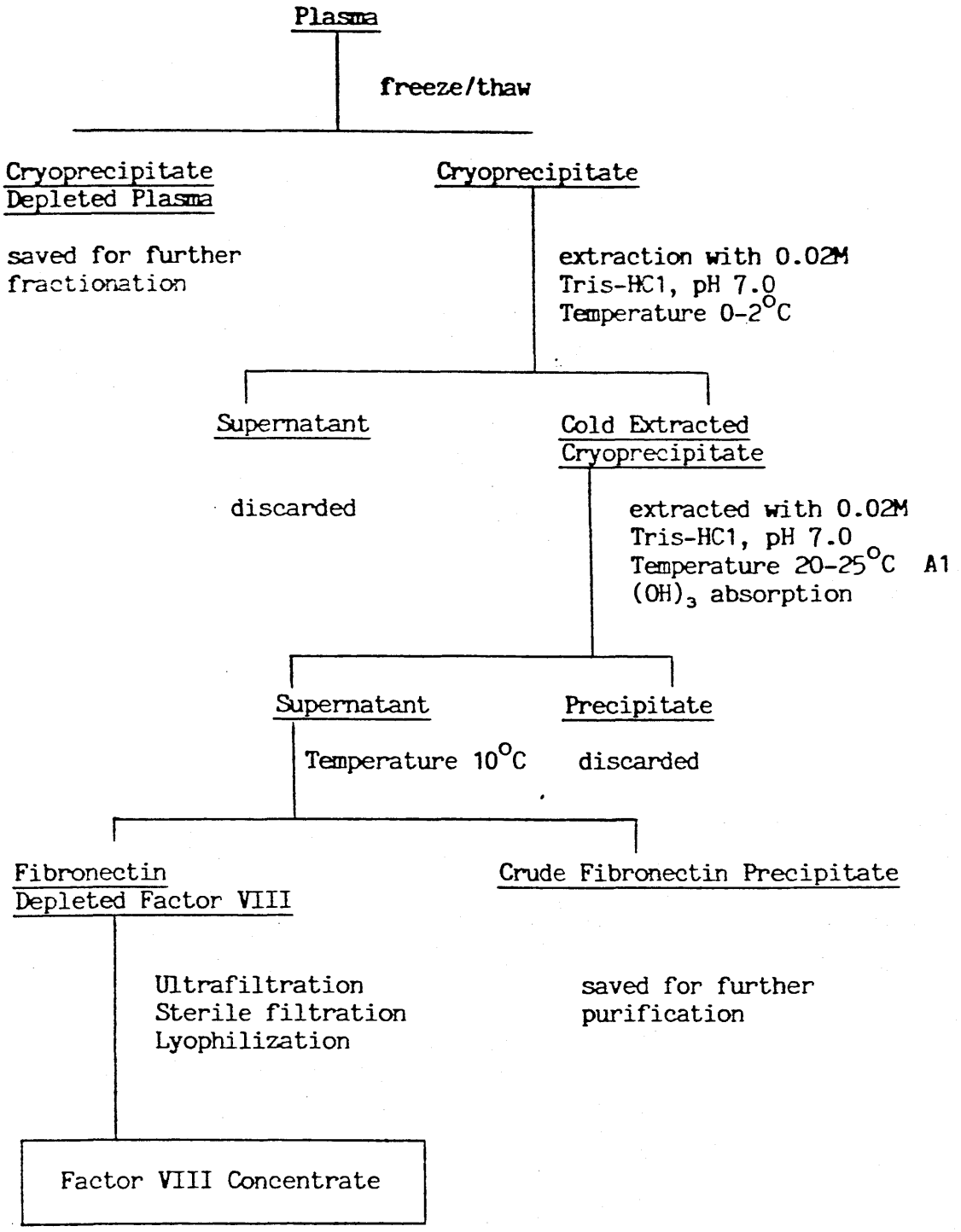


Figure A III.I: Preparation of Factor VIII Concentrate.

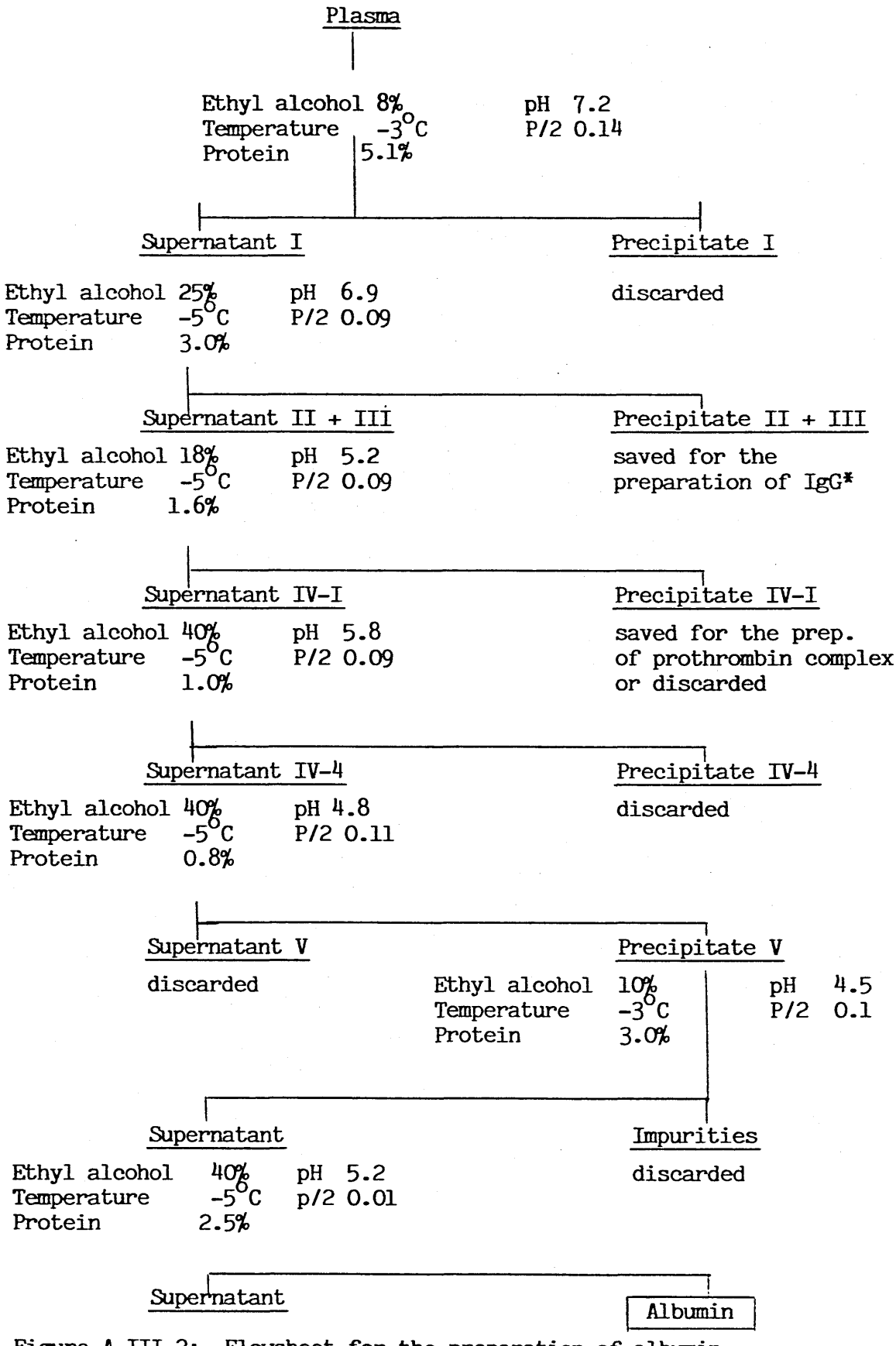


Figure A III.2: Flowsheet for the preparation of albumin.

The parameters of ethyl alcohol concentration, temperature, protein concentration, Ph and ionic strength (P/2) are given for each fractionation.

* IgG - IgG immunoglobulin

hepatitis and limited clinical limitations.

Fractions II and III are sub-fractionated to prepare immunoglobulin. In addition proteins such as lipoproteins, plasminogen, prothrombin and isoglutinins may also be recovered, but only immunoglobulin is currently prepared as a licensed clinical product.

Fraction IV-4 is discarded and Fraction V is used to prepare albumin solutions for clinical use.

Before the final product is prepared for clinical use, the various concentrates obtained from Cohn fractionation must be treated to remove any alcohol which remains in the terminal fraction as an unwanted contaminant at a concentration which may affect the solubility and stability of the product. In addition to ethanol, water may be present as a dilutent in excess of what is required in the final solution and so treatment is also required to remove this water and raise the protein concentration to specified concentrations. The process of thin film evaporation is used to remove unwanted alcohol and water from the protein concentrations. The solution to be treated is spread under vacuum and flows over a supporting surface to which heat is applied. The conditions promote a high rate of evaporation in a system which is designed to maintain rapid transfer of heat without imposing excessive temperature gradients while operating

continuously.

Finally, the concentrates are filtered to produce products which are free of contaminants such as lipids, denatured proteins and micro-organisms.

APPENDIX IV.

BALANCE STUDIES FOR ALUMINIUM, CHROMIUM, MANGANESE AND IRON USING 'NEW CLEANED UP' SOLUTION

Patient:	C.R.
Date of Plasma Exchange	15th June 1989
Diagnosis:	Guillain-Barre
Total Volume of Plasma Exchange:	2.8 litres
Batch No. of albumin 4.3%	331182710

METAL INPUT

	Aluminium	Chromium
Metal concentration:	0.7(umol/l)	504(nmol/l)
Total amount infused:	1.96(umol/l)	1411.2(nmol/l)

	Manganese	Iron
Metal concentration:	174(nmol/l)	41(umol/l)
Total amount infused:	487.22(nmol/l)	114.8(umol/l)

METAL OUTPUT IN EXCHANGE FLUID

Volume of Exchange Fluid = 2.4 l

	Aluminium	Chromium
Metal concentration:	0.5(umol/l)	575(nmol/l)
Metal output in exchange fluid:	1.23(umol/l)	1414.5(nmol/l)

	Manganese	Iron
Metal concentration:	29.7(nmol/l)	12(umol/l)
Metal output in exchange fluid:	73(nmol/l)	29.5(umol/l)

METAL OUTPUT IN URINE

Pre Plasma Exchange

Urine Volume = 1.5 l

	Aluminium	Chromium
Urinary metal concentration:	1.96(umol/l)	6.4(nmol/l)
Total urinary metal excretion:	2.94(umol)	9.6(nmol)

	Manganese	Iron
Urinary metal concentration:	7(nmol/l)	0.2(umol/l)
Total urinary metal excretion:	10.5(nmol)	0.3(umol)

Post Plasma Exchange

Day 1.

Urine Volume = 1.32 l

	Aluminium	Chromium
Metal concentration:	1.80(umol/l)	4.8(nmol/l)
Total concentration:	2.34(umol)	6.2(nmol)
*Net metal excreted:	0	6.2(nmol)

* NB: Net metal excreted = Metal conc. Post exchange - Metal conc. Pre exchange.

	Manganese	Iron
Metal concentration:	7 (nmol/l)	0.2 (umol/l)
Total concentration:	9.1 (nmol)	0.3 (umol)
Net metal excreted:	0	0

Day 2

Urine Volume = 1.2 l

	Aluminium	Chromium
Metal concentration:	1.6 (umol/l)	1.9 (nmol/l)
Total concentration:	1.92 (umol)	2.3 (nmol)
Net metal excreted:	0	1.3 (nmol)

	Manganese	Iron
Metal concentration:	7 (nmol/l)	0.2 (umol/l)
Total concentration:	8.4 (nmol)	0.2 (umol)
Net metal excreted:	0	0

Total Urinary Metal Excreted (day 1 and 2)

Aluminium	Chromium	Manganese	Iron
0	7.5 (nmol)	0	0

CITRATE BALANCE STUDIES

INPUT

Albumin

Citrate level: 0.1 mmol/l

Citrate load infused = 2.8 x 0.1 = 0.28 mmol

OUTPUT

A. Plasma Bag:

Citrate: 0.1 mmol/l

Citrate removed: 0.25 mmol

B. Urine Output:

Urine Output Pre-Exchange

	Citrate concentration	1.29mmol/l
	Basal Output	1.94 mmol

Urine Output Post Exchange

Day 1	Citrate concentration	1.20 mmol/l
	Citrate Output	1.44 mmol
Day 2	Citrate concentration	1.35 mmol/l
	Citrate Output	1.69 mmol

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