

5.0 INVESTIGATOR'S BROCHURE

Sponsors: Gervasio A. Lamas, MD
Mount Sinai Medical Center

Product: Edetate disodium USP

Research Number: 1 U01 AT01156-01

Name(s): Chemical, generic: edetate disodium USP

Edition Number: Version 2

Replaces Previous Edition Number: Version 1

Date: March 14, 2005

Signature Page

Gervasio A. Lamas, MD

Confidentiality Statement

The information contained in this document, especially unpublished data, is the property of Mount Sinai Medical Center and is therefore provided to you in confidence as an investigator, potential investigator, or consultant, for review by you, your staff, and an applicable Institutional Review Board/Independent Ethics Committee. It is understood that this information will not be disclosed to others without written authorization from Dr. Gervasio A. Lamas, Principal Investigator, except to the extent necessary to obtain written informed consent from those persons to whom the drug may be administered.

Investigator’s Brochure – Table of Contents

Table of Contents.....	4
IB 1.1 Summary.....	5
IB 1.1.1. Chelation Therapy in the United States	5
IB 1.1.2 Edetate Disodium USP	5
IB 1.2 Physical, Chemical, and Pharmaceutical Properties and Formulation of Edetate disodium USP	6
IB 1.2.1 Edetate Disodium USP: Product/Formulation.....	6
IB 1.2.2 Edetate Disodium USP Structural formulae (of active ingredient)	7
IB 1.2.3 Formulation of Edetate Disodium USP for TACT	8
IB 1.2.4 Instruction for Storage and Handling.....	8
IB 1.3 Previous Non-clinical Studies of Edetate Disodium USP	8
IB 1.4 Previous Clinical Studies of Edetate Disodium USP.....	9
IB 1.4.1 Case Reports and Case Series	9
IB 1.4.2 Randomized Trials.....	11
IB 1.4.2.1 Reports of Human Toxicities in PACT.....	12
IB 1.4.3 Reports of Human Toxicity in Randomized Trials.....	17
IB 1.4.4 Reports of Human Toxicity in Case Reports and Case Series.....	19
IB 1.4.5 Specific Human Toxicities.....	19
IB 1.4.5.1 Renal Toxicity.....	19
IB 1.4.5.2 Hypocalcemia	20
IB 1.4.5.3 Hypoglycemia.....	21
IB 1.4.5.4 Hypotension	21
IB 1.4.5.5 Trace Metal and Vitamin Deficiency Syndromes.....	21
IB 1.4.5.6 Local Venous Symptoms	21
IB 1.4.5.7 Clotting Parameters.....	21
IB 1.4.5.8 Febrile Episodes.....	22
IB 1.4.5.9 ECG Changes.....	22
IB 1.4.5.10 Heart Failure (HF) / Fluid Overload	22
IB 1.4.5.11 Pregnancy.....	24
IB 1.4.5.12 Miscellaneous	24
IB 1.5 Summary of Data and Guidance for the Investigator	25
IB Literature Cited	26

IB 1.1 Summary

Edetate disodium USP chelation therapy is a commonly used complementary and alternative medicine (CAM) treatment. The US Centers for Disease Control and Prevention estimate that over 66,000 US patients undergo chelation therapy yearly.⁵⁰ Given that each course of treatment in a single patient may entail 40 infusions, as many as one million infusions yearly may be taking place in the US alone. In spite of its highly prevalent use, the evidence base consists primarily of case reports and case series and only three published randomized trials. This NIH-sponsored Trial to Assess Chelation Therapy (TACT) will provide definitive evidence of chelation therapy's clinical utility or lack thereof in patients with coronary disease, defined by a prior myocardial infarction. Eligible patients will have good renal function, and the ability to tolerate weekly fluid infusions. Patients will be infused with either a 10-component 500 mL treatment solution (including edetate disodium USP, ascorbic acid, heparin, procaine HCl, and other components), or a placebo infusion consisting of 500mL 0.9N NaCl, and 0.47% dextrose (5mL of 50% dextrose in 500 mL). The treatment schedule includes 30 weekly infusions, plus 10 maintenance infusions, for a total of 40 infusions. Patients will also receive vitamin and mineral supplements or their respective placebos for the duration of the trial. Patients will be monitored with frequent blood and urine studies. The principal potential toxicity is renal dysfunction, manifested as a rise in the serum creatinine. This is monitored by frequent measurements of serum creatinine, and the dose of edetate disodium USP adjusted by the Accu-Care Services Pharmacy based on report of serum creatinine. Hypocalcemic symptoms, including tetany, is another potentially serious toxicity that may occur if the infusion is administered too rapidly. Other problems may be less common and include symptoms of hypoglycemia, hypotension, ECG changes, febrile episodes, fluid overload, clotting problems, thrombocytopenia, GI distress, trace element deficiency syndromes (these are supplemented in all patients), and problems with venous access.

IB 1.1.1. Chelation Therapy in the United States

Coronary heart disease (CHD) is by far the leading cause of premature morbidity and mortality in the United States. At present, proven therapies include lifestyle modifications, drugs, and procedures. Despite the availability and underutilization of these proven therapies, many patients seek out and receive alternative therapies, including the commonly used complementary and alternative medicine (CAM) practice called chelation therapy. Chelation therapy, as practiced in the CAM community, involves the intravenous administration of edetate disodium USP, combined with high dose antioxidant vitamin and mineral supplements. Thus, any clinical benefit may be due to the effect of edetate disodium USP chelation, high dose antioxidant vitamins and mineral supplements, or both. It has been estimated that in the last few years, over one million patients received more than 20 million infusions¹ “with no serious adverse effects”, but this has not been well-documented. A more recent CDC survey concluded that 66,000 patients yearly use chelation therapy.⁵⁰

IB 1.1.2 Edetate Disodium USP

The pharmacologic effects of edetate disodium USP are due to formation of chelates with divalent and trivalent metals. A stable chelate will form with any divalent metal, a feature shared

by lead, zinc, cadmium, manganese, iron and mercury. The amounts of manganese and iron mobilization are not significant. Copper¹ is not mobilized and mercury is unavailable for chelation because it is too tightly bound to body ligands or it is stored in inaccessible body compartments. The excretion of calcium by the body is not increased following intravenous administration of edetate disodium USP, but the excretion of zinc is considerably increased.¹ Transient reduction of serum calcium can be observed following intravenous (IV) infusion of edetate disodium USP. 1gm of edetate disodium USP can effectively bind approximately 120mg of calcium. In blood, all edetate disodium USP is found in plasma, and is distributed primarily in the extracellular fluid with only about 5% of the plasma concentration found in the spinal fluid. Edetate disodium USP does not appear to penetrate cells. Edetate disodium USP is excreted primarily by the kidneys, with about 50% in one hour and over 95% within 24 hours and is poorly absorbed from the gastrointestinal tract.² Almost none of the compound is metabolized. The half life of edetate disodium USP is 20 to 60 minutes.

IB 1.2 Physical, Chemical, and Pharmaceutical Properties and Formulation of Edetate disodium USP

IB 1.2.1 Edetate Disodium USP: Product/Formulation

Edetate disodium USP³
Chealamide®, Disotate®, Endrate®, Meritate™

Classifications:

- Heavy Metal Antagonists/Chelating Agents
- Toxicology Agents

Description: Edetate disodium, a chelating agent with affinity for divalent and trivalent metals, is indicated in the treatment of hypercalcemia. Because the drug can decrease blood calcium levels too rapidly (causing tetany, cardiac arrhythmias, and respiratory arrest), it now is only rarely used. Due to its pharmacodynamic actions that oppose those of the cardiac glycosides, edetate disodium was once used to treat arrhythmias associated with digitalis toxicity, but digoxin immune Fab is now the preferred agent for this condition. Edetate disodium USP should not be confused with its calcium salt (calcium edetate), which is used to treat lead toxicity. Edetate disodium was approved by the FDA for clinical use in 1956. Edetate disodium chelates and enhances the excretion of other trace metals including magnesium and zinc, and although the drug does not chelate potassium, the administration of edetate disodium can increase renal excretion and decrease serum concentration of this mineral, possibly producing hypokalemia. At present, edetate disodium is used by complementary and alternative medicine practitioners to treat atherosclerosis. The hypothesized mechanism is the chelation of iron and copper, thereby impairing the generation of free radicals.⁴ This mechanism has not been verified. The use and mechanism of action of edetate disodium for this condition remains speculative and is the subject of the present IND application.

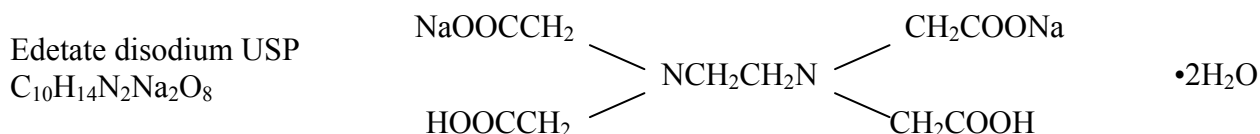
Mechanism of Action: Edetate disodium preferentially binds calcium ions, forming a stable, soluble complex that is then excreted by the kidneys. Serum calcium levels decrease quickly

following intravenous administration of edetate disodium, and 1 gm of edetate disodium has the potential to combine with 120 mg of calcium. The decrease in calcium precipitated by administration of the drug antagonizes the inotropic and chronotropic effects that digitalis glycosides exert on the ventricles of the heart, thereby helping to control digitalis-induced ventricular arrhythmias. In addition, edetate disodium exhibits negative cardiac inotropic activity. A too rapid decrease in serum calcium induced by edetate disodium can precipitate hypocalcemic tetany, seizures, severe cardiac arrhythmias, and respiratory arrest, but these effects are usually related to high doses or rapid infusion rates. Slow IV infusion allows time for calcium in bone to be mobilized to replenish the serum calcium pool, thereby lowering the risk of developing these adverse effects. The concentration of calcium ions in the cerebrospinal fluid is not affected by edetate disodium administration.

Edetate disodium also chelates and enhances the excretion of other trace metals including magnesium and zinc, and although the drug does not chelate potassium, the administration of edetate disodium can increase renal excretion and decrease serum concentration of this mineral, possibly producing hypokalemia. At one time, it was thought the calcium-lowering actions of edetate disodium could be utilized in the management of atherosclerosis but this mechanism has been refuted.⁵ Edetate disodium is still advocated as a treatment for this condition however and its proponents hypothesize that the drug works by chelating iron and copper, thereby impairing the generation of free radicals.⁶ This mechanism has not been verified. The use and mechanism of action of edetate disodium for this condition remains speculative.

Pharmacokinetics: Edetate disodium is administered intravenously, and although the distribution of the drug has not been established, it does not appear to cross the blood-brain barrier to a significant extent. No metabolism of the drug occurs, and an intravenous dose is excreted primarily as the calcium chelate. Approximately 95% of a dose is excreted in the urine within 24 hours. Impaired renal function can reduce the excretion of edetate disodium, possibly leading to nephrotoxicity.

IB 1.2.2 Edetate Disodium USP Structural formulae (of active ingredient)



IB 1.2.3 Formulation of Edetate Disodium USP for TACT

Infusate edetate disodium USP for TACT consists of 500ml of sterile water with the following additives:

Additive	Role of Additive
Up to 3 grams of disodium EDTA	To reduce local discomfort and replace losses
2 grams of magnesium chloride	To reduce local discomfort
100 mg of procaine HCL	To reduce local phlebitis
2500 units of heparin	Anti-oxidant and to achieve isoosmolarity
7 grams of ascorbate	To replace losses
2 mEq KCl	To act as a buffer and reduce discomfort
840 mg sodium bicarbonate	For anti-oxidant properties
250mg pantothenic acid	For anti-oxidant properties
100mg of thiamine	To replace chelation losses
100mg of pyridoxine	
QS with sterile water to 500ml	

The dose of edetate disodium USP is reduced in patients with a smaller lean body mass or reduced renal function as described in section 4.3.1.

IB 1.2.4 Instruction for Storage and Handling

Edetate disodium USP solution prior to mixing in the infusion bag must be stored in a refrigerated environment, and maintained between 2–8° C (or 36-46° F). It must be mixed and infused within 72 hours of preparation.^{7,8} Following mixing in the infusion bag, it must be kept refrigerated if not infused immediately. If not infused immediately, must be stored in a refrigerated environment, and maintained between 2–8° C (or 36-46° F), but must be infused within 24 hours after mixing so long as the original 72 hour limit is not exceeded. If the time or temperature guidelines are violated, the solution should be discarded and not infused.

IB 1.3 Previous Non-clinical Studies of Edetate Disodium USP

Animal data on edetate disodium USP were reported prior to US marketing. The LD₅₀ varied from 500 to 7000 mg/kg/day, depending on the species, route, and mode of administration.⁹ Early toxicology detected that rapid administration could dangerously lower calcium levels. In animals, the rapid induction of severe hypocalcemia could result in tetany, seizures, and death.¹⁰ However, within a decade of the introduction of edetate disodium USP for human use, it became accepted that infusion rates below 20mg/min would not produce symptomatic hypocalcemia.⁹ A maximum infusion rate of 17mg/min will be used in TACT.

In 1953 a study¹¹ reported the effects of edetate on supplementary cholesterol in 34 male rabbits. Rabbits were fed crystalline cholesterol daily, and were either subcutaneously injected with up to

3 grams of edetate disodium 3-5 days per week or orally administered 3 grams of edetate dicalcium. Rabbits receiving the injected or oral dosage of edetate had normal blood cholesterol levels compared to the higher levels of blood cholesterol observed in the untreated group.

A study on the toxicity of edetate disodium USP in male and female rats was reported in 1967.¹² 300-500 mg/kg of CaNa₂Edetate was administered to 109 rats and a normal saline solution was administered to 97 rats. Each solution was given intraperitoneally each day for 10 consecutive days. There were no significant increases in serum creatinine or urea nitrogen observed in the rats receiving the edetate disodium USP solution. The chelate was eliminated within 24 hours of administration.

Another study¹³ in 1990 reported the results on aortic calcium levels in 26 rabbits receiving a high versus low cholesterol diet, and infusions of edetate disodium versus a saline solution. A marked reduction in plaques of induced atherosclerosis was observed in the groups receiving the edetate disodium infusions, while calcified plaques were present in the groups that did not receive edetate disodium.

IB 1.4 Previous Clinical Studies of Edetate Disodium USP

Studies on human experience with edetate disodium USP, particularly for the treatment of CHD, indicate some toxicities, and a few adverse events. A review of research related to human toxicities resulting from the use of edetate disodium USP is presented below.

IB 1.4.1 Case Reports and Case Series

The majority of the clinical literature that reports the benefits of chelation therapy is in the form of case reports and case series.¹⁴ Most case series report on an individual practitioner's clinical practice. Cranton¹⁵ reports that by 1993, there were more than 4600 documentary outcome reports supporting chelation therapy. These studies may be interpreted to suggest a striking benefit of chelation therapy; however, most do not have control groups, patient selection criteria are overly broad, measurements of endpoints are inconsistent, and follow up is incomplete. A cautious interpretation of this literature suggests that there are ample suggestions of benefit, but clear evidence is lacking. For the sake of brevity, only 3 representative studies spanning nearly 3 decades will be reviewed here; selected others are listed in Table IB.1 below.

In 1963, **Kitchell and co-workers**¹⁶ reported on 28 patients with severe angina who underwent chelation therapy. Patients were monitored with exercise testing (fixed speed and inclination treadmill and Master's two-step). The authors concluded that early after chelation, there was little improvement. However, within 3 months of therapy, about 60% of patients reported improvement based both on patients' impression and their documented exercise tolerance. Nonetheless, the benefit was not felt to be long-lasting.

Olszewer and Carter¹⁷, in 1988, reported a retrospective analysis of 2870 patients treated at a private clinic in Sao Paulo, Brazil, who underwent chelation therapy between May 1983 and September 1985. Patients received a total of approximately 81,000 infusions. The protocol used was that recommended by the American Academy of Medical Preventives; and consisted of

edetate disodium USP 50 mg/kg body weight given over 3 - 3.5 hours. Additives included vitamin C, B complex, and magnesium. There were 120 patients lost to follow up who were censored from the report. Treatments were given 2-3 times weekly. General lifestyle advice and oral multivitamins were also administered. Cardiac disease was present in 29.4%, peripheral vascular disease in 39.4%, cerebrovascular and degenerative disease of the CNS in 17.7%, scleroderma in 0.1%, and other geriatric vascular diseases in 13.4%. Of the 844 patients who had a diagnosis of ischemic heart disease, most (57.6%) had coronary insufficiency without infarction, 27.6% coronary insufficiency and infarction, and 4.8% coronary insufficiency with other complications. The authors report that 76.9% of patients had a marked improvement, defined as a positive stress test that subsequently became negative after a course of chelation therapy.

Casdorph¹⁸, in 1981, reported a case series of 18 patients in whom left ventricular ejection fraction was measured with radionuclide ventriculography preceding and following 20 weekly 3-hour infusions of 3 grams of edetate disodium USP in 250 cc of lactated Ringer's with 200 mg of lidocaine added to the infusate. The average improvement in ejection fraction was 5.8% (range - 2% to 16%) and was highly significant ($p < 0.001$).

Table IB.1 Summary of Case Series

First author (year)	Sample Size	Outcome measures	Result
Clarke (1955) ¹⁹	22	Symptoms	some improvements
Clarke (1956) ²⁰	20	Symptoms	19 improved, 1 died
Boyle (1957) ²¹	20	Symptoms, ECG	significant improvements
Meltzer (1960) ²²	10	Symptoms, ECG	9 improved
Clarke (1960) ²³	76	Symptoms	58 improved
Kitchell (1961) ²⁴	10	Symptoms	9 improved
Boyle (1961) ²⁵	10	Symptoms, ECG	9 improved
Meltzer (1961) ²⁶	81	not stated	"effective"
Kitchell (1963) ^{16*}	28	Symptoms, ECG	18 improved,
Lamar (1964) ²⁷	15	Symptoms	15 improved
Lamar (1966) ²⁸	3	Symptoms	1 improved, 1 died
Evers (1979) ²⁹	3000	Symptoms	>90% improved
Casdorph (1981) ^{18*}	18	ejection fraction	17 improved

Robinson (1982) ³⁰	248	Symptoms, ECG	significant improvements
Olszewer (1988) ^{17*}	844	Symptoms	821 improved
McGillen (1988) ³¹	1	Angiography	No evidence of benefit
Wirebaugh (1990) ³²	1	Angiography	No evidence of benefit
Deycher (1992) ³³	215	Symptoms	70% improvement
Hancke (1992) ³⁴	42	Need for surgery	39 cancelled surgery
Hancke (1993) ³⁵	470	Symptoms	Significant improvements

*Studies discussed in above text

In most of these case reports and series, severe adverse events were not reported.

IB 1.4.2 Randomized Trials

Only 3 randomized trials of edetate disodium USP chelation for patients with atherosclerotic vascular disease have been conducted. A fourth trial, our Pilot to Assess Chelation Therapy (PACT), has been reported as an abstract, and we include safety data of patients in the chelation arm.

The first trial by Guldager et al³⁶ enrolled 159 patients with stable intermittent claudication for at least 12 months, and excluded patients with underlying conditions such as renal insufficiency, cardiac disease, or diabetes. The treatment regimen consisted of 20 infusions administered over 5 to 9 weeks. Patients also received oral supplements of multivitamins and magnesium. Findings indicate no differences in any parameters studied in the edetate disodium USP treated group compared to placebo/control group.

Van Rij³⁷ reported the second trial in 1994. This trial included 32 patients with peripheral vascular disease confirmed by angiography. Diabetics were excluded, and patients were required to stop smoking. The active infusion consisted of 3.0 g of edetate disodium USP, 0.76g magnesium chloride, and 0.84 g sodium bicarbonate in normal saline, to a total volume of 500 ml. The placebo infusion was 500 ml of normal saline. Both groups received parenteral vitamin supplements. There were no significant differences reported in pain-free walking distance, or total walking distance when the edetate disodium USP treated group was compared to the placebo group. At 3 months after treatment, however, resting ankle-brachial index showed some improvement in the chelation group in both legs, with a significant between-groups effect favoring chelation. An extensive analysis of quality of life also was performed, with mixed results. Although there were no differences in scales measuring general health and effect of poor circulation on life activities, chelation patients scored better on 2 scales that rated the level of physical activity ($p < 0.05$ for between-groups differences) 3 months after therapy.

Knudtson and colleagues³⁸ carried out the Program to Assess Alternative Treatment Strategies to Achieve Cardiac Health (PATCH), a 6-month randomized trial that measured exercise capacity

in 84 stable angina patients randomized to receive either edetate disodium USP treatment or placebo. Patients were eligible to participate in the trial if they were over the age of 21, had proven CAD, stable angina pectoris, and ≥ 1 mm ST-segment depression within 2-14 minutes on a gradually ramping treadmill test. There were 39 patients ultimately randomized to each treatment group, receiving 40 mg/kg of edetate disodium USP up to a maximum of 3 g, or placebo. Both were administered in an IV saline solution over a 3-hour period, 2 times per week over 15 weeks, then once per month for 3 months, for a total of 33 treatments. All patients were given oral multivitamins. There were no significant differences in clinical outcomes between the treatment groups. There were no deaths, 2 MIs (1 in the chelation group and 1 in the placebo group), and 15 hospitalizations for worsening angina (9 in the chelation group and 6 in the placebo group). Both groups were able to increase their exercise times approximately 1 minute, an improvement that the investigators attributed to placebo or "training" effect. The investigators concluded that a trial of far larger sample size was necessary to reach any definitive conclusions. The present investigators have been given access to confidential renal function safety data, reported in Table IB.7.

The Pilot to Assess Chelation Therapy (PACT) was a 40-patient randomized trial of chelation therapy versus placebo, with change in endothelium-dependent, flow-mediated brachial artery dilation as its primary endpoint, in patients who fulfill the TACT entry criteria. The chelation protocol consisted of 15 weekly infusions of chelation therapy prepared with the published ACAM protocol. The placebo group received 15 infusions of normal saline. The methodology and algorithms for calculating and adjusting edetate disodium USP dose were identical to those of TACT. All patients received a low-dose vitamin supplementation regimen. The total cohort of 40 patients has been enrolled (chelation=30 patients; placebo =10 patients). Although the study has not yet been published in full, we report confidential safety data in the 30 chelation patients, presented below. PACT provides reassuring data on blood pressure, pulse, renal, electrolyte, calcium and hematological parameters.

IB 1.4.2.1 Reports of Human Toxicities in PACT

The Pilot to Assess Chelation Therapy (PACT) was an ongoing 40-patient randomized trial of chelation therapy versus placebo, with change in endothelium-dependent, flow-mediated brachial artery dilation as its primary endpoint, in patients who fulfilled the TACT entry criteria. The chelation protocol consisted of 15 weekly infusions of chelation therapy prepared with the published ACAM protocol. The placebo group received 15 infusions of normal saline. The methodology and algorithms for calculating and adjusting edetate disodium USP dose were identical to those of TACT. All patients received a low-dose vitamin supplementation regimen. A total of 40 patients were enrolled (chelation=30 patients; placebo =10 patients). Although the study has not yet been published in full, we provide safety data in the 30 chelation patients. PACT provides reassuring data on blood pressure, pulse, renal, electrolyte, calcium and hematological parameters. Data collected show little change in laboratory values over 14 infusions (see Tables IB.2, IB.3, and IB.4). In PACT, 1 patient was found to have a significant increase in AST and ALT (See Table IB.5 for a list of adverse events in PACT).

Table IB.2
Safety Measurements for PACT
30 Patients Randomized to Chelation

Infusion #	Creatinine	Glucose	Hematocrit	Magnesium	Platelet Count	Potassium
Baseline	1.1 ± 0.2	99.4 ± 34.1	40 ± 8.5	1.9 ± 0.5	203.8 ± 69.3	4.4 ± 0.6
2	1.1 ± 0.2	117.1 ± 34.1	40.7 ± 3.4	1.9 ± 0.5	207.8 ± 57.4	4.4 ± 0.6
5	1.1 ± 0.2	113.7 ± 38.6	40.0 ± 3.8	2.1 ± 0.3	202.8 ± 55.2	4.4 ± 0.6
10	1.0 ± 0.2	109.2 ± 34.8	39.2 ± 3.8	1.8 ± 0.5	201.9 ± 40.0	4.4 ± 0.6
14	1.1 ± 0.2	113.2 ± 36.9	40.0 ± 4.2	2.1 ± 0.3	211.3 ± 47.3	4.6 ± 1.0

Table IB.3
Initial and Final Safety Measurements For PACT
10 Patients Randomized at MSMC to Chelation

Patient #	Calcium		AST		ALT	
	Initial	Final	Initial	Final	Initial	Final
1	9.5	9.2	-	-	-	-
2	10.4	9.8	27	32	33	35
3	9.4	9.3	-	-	-	-
4	9.3	9.4	17	21	23	24
5	9.7	9.6	27	29	23	25
6	9.0	8.9	20	21	14	19
7	9.2	10	22	66	20	130
8	9.6	9.7	19	22	27	28
9	8.8	8.5	-	-	-	-
10	8.9	8.9	27	22	32	27
Total Mean ±SD	9.38 ± 0.47	9.33 ± 0.47	21.89 ± 4.27	30.42 ± 16.28	24.57 ± 6.70	41.14 ± 39.48

Table IB.4
Systolic BP For PACT
30 Participants Randomized to Chelation

Infusion Number	Systolic BP (Mean±SD)		
	Pre-Infusion	During Infusion	Post-Infusion
1	126.7 ± 15.8	122.4 ± 15.0	126.4 ± 19.0
2	120.9 ± 15.7	120.9 ± 15.7	121.8 ± 20.0
3	122.8 ± 14.1	114.6 ± 24.4	119.6 ± 17.5
4	125.8 ± 18.0	122.1 ± 15.1	126.2 ± 19.4
5	124.7 ± 19.8	121.8 ± 18.0	121.3 ± 14.5
6	117.2 ± 14.9	119.3 ± 17.3	124.4 ± 18.3
7	117.2 ± 14.2	124.7 ± 20.7	124.9 ± 16.3
8	123.4 ± 18.0	121.5 ± 16.7	124.2 ± 16.2
9	124.5 ± 18.4	121.6 ± 16.8	123.4 ± 19.6
10	123.3 ± 25.6	124.6 ± 25.0	125.5 ± 22.4
11	120.9 ± 19.0	121.3 ± 17.4	121.4 ± 17.9
12	120.4 ± 18.7	115.3 ± 14.1	117.4 ± 12.3
13	123.2 ± 18.4	122.3 ± 18.9	122.7 ± 15.6
14	125.6 ± 17.7	120.4 ± 14.8	119 ± 16.3
15	123.0 ± 16.4	119.4 ± 17.8	121.6 ± 15.6

Table IB.5
Summary of Adverse Events in PACT

	# of Events
Cessation for doubling of baseline creatinine or rise in creatinine >2.5	0
Fall in platelets below normal range	1
Cessation of heparin	2
Hypocalcemia	0
Hypoglycemia	0
Doubled Liver Enzymes	1
Progressive angina and cardiac catheterization	1
AF, Bradycardia, and Heart Failure	1
Bladder Tumor	1
Diverticulitis	1
Hematoma at the site of infusion	1

IB 1.4.3 Reports of Human Toxicity in Randomized Trials

In the randomized trials, the laboratory data are collected in an unbiased and blinded fashion and are available for three of the four published trials. In the Guldager study,³⁶ adverse event data were reported on the 153 randomized patients. In PATCH, with the cooperation of the investigators, we have secured further unpublished laboratory data on creatinine for their 84 randomized patients. Finally, in PACT, we unblinded the data for the 30 patients who have undergone chelation therapy. In the van Rij study,³⁷ adverse events were not reported.

In these three randomized trials, as shown in Table IB.6, the data on adverse event are reassuring for worsening angina, vascular events, cardiac arrhythmia, fatigue/faintness, GI symptoms, hematologic abnormalities, renal insufficiency, phlebitis at the infusion site, hypocalcemia, pain, and other miscellaneous reported events.

Based on these data, and understanding that the randomized trials evidence is most reliable, we expect a low rate of adverse events and an overall safe intervention.

Table IB.6
Peer Reviewed Literature: Randomized Trials of Chelation for CVD

Author (citation)	Sample Size	Entry Criteria	Endpoints	Adverse Events										
				Worsening angina	Vascular event	Cardiac Arrhythmia	Fatigue/faintness	GI symptoms	Hematologic Abnormalities	Renal Insufficiency	Phlebitis at infusion site	hypocalcemia	Pain	Other
Guldager B, et al Journ of Int Med 1992;231:261-267.	153	intermittent claudication	pain-free walking, ABI		stroke 1 chelation		23 chelation, 12 placebo	11 chelation, 7 placebo		7 chelation, 9 placebo	35 chelation, 28 placebo	6 chelation, 2 placebo	headache 1 chelation	1 chelation - dermatitis
PATCH Knutson ML et al JAMA 2002;287(4):481-	84	proven CAD	ischemia by ECG	9 chelation, 6 placebo	MI 1 chelation, MI 1 placebo			1 placebo		1 chelation			lower back 1 placebo	1 placebo - gout
PACT (unpublished)	30 Chelation Patients	post myocardial infarction	MI, stroke, or death		1 underwent catheterization			0		1 thrombocytopenia	0	0	1 pack pain	1 doubled liver enzymes

Blank=not reported

No adverse events reported:

van Rij AM, et al Circ 1994;90:1194-1199.

IB 1.4.4 Reports of Human Toxicity in Case Reports and Case Series

The peer-reviewed literature includes case series and case reports of chelation therapy for CVD as well as for lead poisoning for which edetate was approved by the FDA. Adverse events were not reported for many of the case series and case reports. Furthermore, the interpretability is limited by the design as well as the lack of standardized protocols and uniformity of patient entry criteria.

IB 1.4.5 Specific Human Toxicities

IB 1.4.5.1 Renal Toxicity

The most important potential adverse event from administration of salts of edetate is renal toxicity. Holland³⁹ in 1953 described 5 patients treated with very large, rapidly administered doses of edetate disodium USP for hypercalcemia of malignancy. For example, one patient received 20g edetate disodium USP over 15 minutes. One patient died directly as a result of the infusion, and 2 others had some degeneration of renal cells. Overall, the reports of nephrotoxicity mostly focus on edetate disodium USP treatment for hypercalcemia or lead intoxication, conditions with independent reasons for renal failure. Indeed, Doolan⁴⁰ and Foreman⁴¹ found that nephrotoxicity required the administration of 300-500 mg/kg/day for 10 days and 203mg/kg/day for 16 days, respectively – doses far higher than will be used in TACT. Recommended doses of edetate disodium USP have been associated with nephrotoxicity in certain cases. However, like in the case reported by Oliver,⁴² the development of renal failure was made more likely by the presence of underlying renal disease (baseline creatinine of 2.1), and daily administration of edetate disodium USP over 4 weeks with breaks only on weekends. Meltzer⁴³ et al reported 2000 infusions given on alternate days over 2-years in 81 patients without a single case of nephrotoxicity. McDonagh⁴⁴ et al reported that among 383 patients treated with 10 infusions, 50% demonstrated an improvement in creatinine, while 34% a mild rise.

In summary, edetate disodium USP can be a nephrotoxic agent, especially in cases of lead poisoning, or in conjunction with other chelating agents, and in the setting of high doses and frequent administration. When administered on a weekly schedule, with intermittent monitoring of creatinine leading to dose adjustment, the rate of renal adverse events is expected to be very low. This is supported by the benign course of creatinine levels of patients receiving chelation therapy in the PATCH randomized trial (Table IB.7).

Table IB.7
Creatinine Levels for PATCH⁴⁵
84 Participants Randomized to Chelation

Infusion #	Creatinine (Mean±SD)
Baseline	0.91 ± 0.19
5	0.90 ± 0.21
10	0.89 ± 0.22
15	0.89 ± 0.21
20	0.91 ± 0.20
25	0.89 ± 0.22
30	0.91 ± 0.21
33	0.91 ± 0.17

In TACT, renal function is measured 10 times during the infusion phase, and dipstick urinalysis is recorded 4 times. The dose of edetate disodium USP is adjusted based on creatinine clearance, and stopping for a doubling of creatinine or exceeding a creatinine of 2.5 mg/dl is built in to the clinical and pharmacy protocols.

IB 1.4.5.2 Hypocalcemia

Immediate: Rapid infusions of edetate disodium USP can cause tetany, seizures, and death. Thus, careful attention to the TACT infusion regimen so as not to exceed 160 cc/hour is mandatory. Clinical units will be required to have infusions of intravenous calcium gluconate available and will be trained in recognizing and treating hypocalcemia.

Long-term: The mobilization from bones is thought to be due to pulsatile lowering of calcium stimulating the parathyroid to release parathormone thus pulling calcium out of the bones. The kidneys respond by releasing phosphorus and thus stabilize the calcium/phosphorus ratio. Because the release of parathormone is pulsatile, an increase, rather than decrease, of new bone formation occurs¹. Osteoporosis has been monitored in patients undergoing chelation therapy. In one study of 61 patients (38 women), bone densitometry was performed before and after edetate disodium USP chelation therapy. They noted no decrease in actual bone density levels and a slight, though non-significant increase. They noted no gender differences.⁴⁶

IB 1.4.5.3 Hypoglycemia

All preparations of edetate disodium USP may cause hypoglycemia in insulin-requiring diabetics. It is unclear if this is due to an effect on the absorption of the exogenously-administered insulin, or to an effect on glucose tolerance.⁴⁷ Nonetheless, the study protocol calls for diabetics on insulin to snack before the infusion, and for study sites to be able to recognize symptoms of hypoglycemia and have oral and intravenous glucose supplements available for use if necessary. Hypoglycemia has not been observed in the PACT. If hypoglycemia occurs during or after infusions despite compliance with the advice to snack before infusions, diabetics will be requested to reduce the dose of their morning insulin by 50% on infusion days, and their primary physician will be notified.

IB 1.4.5.4 Hypotension

A fall in systolic blood pressure >20mmHg may rarely be observed. Meltzer reported it during 33 of 2000 infusions (1.75%). In PACT, during 1 out of 395 infusions, a patient experienced transient hypotension, which resolved within 15 minutes. This did not recur in the same patient during subsequent infusions and was not experienced by any of the other patients (Table 6).

IB 1.4.5.5 Trace Metal and Vitamin Deficiency Syndromes

The principal B-vitamin deficiency syndrome reported has been related to skin rashes and glossitis, and has been responsive to repletion of pyridoxine. The TACT infusion regimen and the supplements taken by all participants include pyridoxine supplements. Zinc excretion has been found to increase more than 20-fold following edetate disodium USP chelation. Despite the absence of clear evidence that a zinc-deficiency syndrome exists in association with chelation therapy, the current recommendations are for zinc supplementation, as is being done in TACT.

IB 1.4.5.6 Local Venous Symptoms

Local symptoms are common in patients receiving multiple infusions. In TACT, patients are ineligible if they do not have venous access. In addition, a small dose of heparin is added to the infusion to prevent phlebitis. Finally, magnesium is added to the infusion to decrease the discomfort. These techniques have proven successful in maintaining the blind in the PACT.

IB 1.4.5.7 Clotting Parameters

There are reports that edetate disodium USP prolongs platelet aggregation in the presence of thrombin.⁴⁸ The present recommended chelation regimen calls for 2500 units of unfractionated heparin with each infusion. As heparin can cause thrombocytopenia, the principal safety parameter to be followed will be platelet count. Heparin will be omitted from the infusion if the platelet count falls below 100,000, or decreases by 50% from baseline.

IB 1.4.5.8 Febrile Episodes

A flu-like syndrome was reported as occurring with edetate disodium USP chelation in the 1950s. However, this has become a rare phenomenon at present. The TACT investigators and coordinators will monitor for and report this syndrome.

IB 1.4.5.9 ECG Changes

Soffer⁴⁹ et al report that edetate disodium USP infusions suppressed ectopic ventricular beats and ventricular tachycardia, slowed sinoatrial node discharge, enhanced AV nodal automaticity, and increased the automaticity of ventricular foci during complete heart block. Some investigators have reported T-wave changes, and still others increased heart rate without T wave changes. PACT did not detect significant changes in heart rate/pulse as is reported below (Table IB.8).

IB 1.4.5.10 Heart Failure (HF) / Fluid Overload

Fluid overload leading to HF is occasionally reported in chelation patients, particularly in those with a prior history of HF. In PACT, one patient developed atrial fibrillation with a slow ventricular rate, and, secondarily, heart failure.

Table IB.8
Heart Rate in PACT
30 Participants Randomized to Chelation

Infusion Number	Pulse (Mean±SD)		
	Pre-Infusion	During Infusion	Post-Infusion
1	64.9 ± 9.3	63.2 ± 8.8	64.2 ± 8.5
2	65.8 ± 10.1	63.3 ± 9.2	63.8 ± 9.0
3	66.8 ± 10.6	64.2 ± 8.7	64.4 ± 8.8
4	68.8 ± 13.5	67.5 ± 13.0	66.5 ± 12.6
5	68.3 ± 10.4	66.6 ± 8.7	66.4 ± 9.0
6	69.0 ± 13.8	65.9 ± 12.6	67.4 ± 12.5
7	67.4 ± 10.6	63.9 ± 9.6	64.0 ± 8.4
8	67.7 ± 8.7	64.3 ± 7.5	65.5 ± 8.0
9	66.7 ± 8.2	63.7 ± 7.0	65.2 ± 8.2
10	67.1 ± 8.1	65.8 ± 6.7	66.0 ± 7.3
11	66.3 ± 8.3	64.0 ± 8.5	65.6 ± 8.4
12	67.5 ± 9.8	64.0 ± 7.3	64.5 ± 8.7
13	65.9 ± 7.8	63.6 ± 8.6	65.0 ± 12.8
14	64.7 ± 8.1	64.7 ± 8.1	63.9 ± 6.2
15	66.3 ± 8.7	65.4 ± 7.8	65.9 ± 9.7

IB 1.4.5.11 Pregnancy

One reproduction study was performed in rats at doses up to 13 times the human dose and revealed no evidence of impaired fertility or harm to the fetus due to EDTA. Another reproduction study performed in rats at doses up to about 25 to 40 times the human dose revealed evidence of fetal malformations, which were prevented by simultaneous supplementation of dietary zinc. There are, however, no adequate and well-controlled studies in pregnant women. The package insert states that animal reproduction studies are not always predictive of human response. Consequently, this drug should be used during pregnancy only if clearly indicated.

IB 1.4.5.12 Miscellaneous

There are a series of miscellaneous symptoms and laboratory abnormalities that have been reported. These include: tremors, headache, numbness, tingling, cheilosis, nausea, vomiting, anorexia, excessive thirst, mild increases in SGOT and SGPT (ALT and AST), histamine-like reactions (sneezing, nasal congestion, lacrimation), rash, transient bone marrow depression, anemia. These reactions are generally both unusual and mild, and will be monitored by laboratory exams and by clinical history.

IB 1.5 Summary of Data and Guidance for the Investigator

Edetate disodium USP chelation therapy is a commonly used complementary and alternative medicine (CAM) practice. Despite its use on many patients over the last decades, the evidence base consists primarily of case reports and case series and only three published and one other small but analyzable randomized trial. This NIH-sponsored Trial to Assess Chelation Therapy (TACT) will provide definitive evidence of the clinical utility of chelation therapy for patients with coronary artery disease, or its lack thereof. Eligible patients will be at least 50 years old, with a history of prior MI, good renal function, and the ability to tolerate weekly fluid infusions. Patients will be infused with either a 10-component 500 mL treatment solution (including edetate disodium USP, ascorbic acid, heparin, procaine HCl, KCl, sodium bicarbonate, magnesium chloride, thiamine, pantothenic acid, and pyridoxine), or a placebo infusion consisting of 500mL of 0.9N NaCl, and 0.47% dextrose. The treatment schedule includes 30 weekly infusions, plus 10 maintenance infusions, for a total of 40 infusions. The schedule of the maintenance infusions is flexible, and may occur as slowly as every 8 weeks for patients randomized early in the trial, to every 5 weeks, for patients randomized late in the trial. TACT will provide an infusion schedule for each patient upon randomization. Patients will also receive vitamin and mineral supplements or their respective placebos for the duration of the trial. Patients will be monitored with frequent blood and urine studies. The principal potential toxicity is renal dysfunction, manifested as a rise in the serum creatinine. This is monitored by frequent measurements of serum creatinine, and the dose of edetate disodium USP is adjusted by the Accu-Care Services Pharmacy based on the report of serum creatinine. Hypocalcemic symptoms, including tetany, is another potentially serious toxicity that may occur if the infusion is administered too rapidly. Other problems may be less common and include symptoms of hypoglycemia, hypotension, ECG changes, febrile episodes, fluid overload, clotting problems, thrombocytopenia, GI distress, trace element deficiency syndromes (these are supplemented in all patients), and problems with venous access.

IB Literature Cited

¹Cranton EM. A Textbook on EDTA Chelation Therapy, 2nd Edition. Charlottesville: Hampton Roads Publishing, 2001.

²Foreman H, Trujillo TT. The metabolism of C¹⁴ labeled ethylene diamine tetra-acetic acid. *J Lab Clin Med* 1954;43:566-71.

³Clinical Pharmacology 2000 Copyright; © 2002 Gold Standard Multimedia, Revised 7/16/01.

⁴Babu AN, Gonzalez-Pena H. Iron chelating agents are not useful in treating atherosclerosis. *Ann Intern Med* 1994;121:384.

⁵Ibid.

⁶Babu AN, Gonzalez-Pena H. Iron chelating agents are not useful in treating atherosclerosis. *Ann Intern Med* 1994;121:384.

⁷Allwood MC. Compatibility and stability of TPN mixtures in Big Bags. *J Clin Hosp Pharm* 1984;9:181-198.

⁸Dahl GB, Jeppsson RI, Tengborn HJ. Vitamin stability in a TPN mixture stored in an EVA plastic bag. *J Clin Hosp Pharm*. 1986;11(4):271-9.

⁹Foreman H. Toxic side effects of ethylene diamine tetra-acetic acid. *J Chron Dis* 1963;16:319-23.

¹⁰Popovici A, Geschickter CF, Reinovsky A, and Rubin M. Experimental control of serum calcium levels in vivo. *Proc Soc Exper Biol Med*. 1950;74:415-417.

¹¹Uhl HS, Brown HH, Zlatkis A, et al. Effect of ethylenediamine tetraacetic acid on cholesterol metabolism in rabbits. *Am J Clin Pathol* 1953;23:1226-33.

¹²Doolan PD, Schwartz SL, et al. An evaluation of the nephrotoxicity of ethylenediaminetetraacetate and diethylenetriaminepentaacetate in the rat. *Tox App Pharm* 1967;10:481-500.

¹³Kaman RL, Rudolph CJ, McDonagh EW, Walker FM. Effect of EDTA chelation therapy on aortic calcium in rabbits on atherogenic diets: Quantitative and histochemical studies. *J Adv Med* 1990;3:13-21.

¹⁴Grier MT, Meyers DG. So much writing, so little science: A review of 37 years of literature on Edetate Sodium chelation therapy. *Ann Pharmacother* 1993; 27:1504-9.

¹⁵Cranton EM and Frackelton JP. Current status of EDTA chelation therapy in occlusive arterial disease. *J Hol Med* 1982;1:24.

- ¹⁶Kitchell JR, Palmon F, Aytan N, Meltzer LE. The treatment of coronary artery disease with disodium EDTA, a reappraisal. *Amer J Cardiol* 1963;11:501-06.
- ¹⁷Olzsewer E, Carter JP. EDTA chleation therapy: a retrospective study of 2,870 patients. *J Adv Med* 1989;2:137-9.
- ¹⁸Casdorph HR. EDTA chelation therapy, efficacy in arteriosclerotic heart disease. *J Hol Med* 1981;3:53-9.
- ¹⁹Clarke NE, Clarke CN, Mosher RE. The in vivo dissolution of metastatic calcium. *Amer J Med Sci* 1955;229:142-49.
- ²⁰Clarke NE, Clarke CN, Mosher RE. Treatment of angina pectoris with disodium ethylene diamine tetraacetic acid. *Amer J Med Sci* 1956;232:654-66.
- ²¹Boyle AJ, Jasper JJ, McCormick H. Studies in human and induced atherosclerosis employing – (EDTA). *Bull Swiss Acad Med Sci* 1957;13:408.
- ²²Meltzer LE, Ural ME, Kitchell JR. The treatment of coronary artery disease with disodium EDTA. *Metal Binding in Medicine*. Philadelphia: JB Lippincott, 1960:132-136.
- ²³Clarke Sr NE, Clarke Jr NE, Mosher RE. Treatment of occlusive vascular disease with disodium ethylene diamine tetraacetic acid (EDTA). *Amer J Med Sci* 1960;239:732-44.
- ²⁴Kitchell JR, Metlzer LE, Seven MJ. Potential uses of chelation methods in the treatment of cardiovascular diseases. *Prog Cardiovasc Dis* 1961;3:338-349.
- ²⁵Boyle AJ, Clarke NE, Mosher RE, McCann DS. Chelation therapy in circulatory and sclerosing diseases. *Fed Proc* 1961;20(Suppl 10):243.
- ²⁶Meltzer LE, Kitchell JR, Palmon Jr F. The long term use, side effects, and toxicity of disodium ethylenediamine tetraacetic acid (EDTA). *Amer J Med Sci* 1961:51-57.
- ²⁷Lamar CP. Chelation therapy of occlusive arteriosclerosis in diabetic patients. *Angio* 1964;15:379-395.
- ²⁸Lamar CP. Chelation endarterectomy for occlusive atherosclerosis. *J Amer Ger Soc* 1966;14:272-294.
- ²⁹Evers R. Chelation of vascular atheromatous disease (experience with 3000 patients). Private Communication 1975; Chelation Bibliography – a collection of 80 articles. *Amer Col Adv Med*.
- ³⁰Robinson DM. Chelation therapy. *NZ Med J* 1982;95:750.

- ³¹McGillen MJ, Mancini GBJ. Inefficacy of EDTA chelation therapy for coronary atherosclerosis. *N Engl J Med* 1988;318:1618-1619.
- ³²Wirebaugh SR, Geraets DR. Apparent failure of edetic acid chelation therapy for the treatment of coronary atherosclerosis. *DICP Ann Pharmacother* 1990;24:22-5.
- ³³Deycher GP. Antioxidant therapy in the aging process. *Free Radicals in Aging*. Basel: Verlag, 1992:93-117.
- ³⁴Hancke C, Flytlie K. Manipulation with EDTA. *Ugeskar Laeger* 1992;154(32):2213-2215.
- ³⁵Hancke C, Flytlie K. Benefits of EDTA chelation therapy in arteriosclerosis: a retrospective study of 470 patients. *J Adv Med* 1993;6:161-171.
- ³⁶Guldager B, Jelnes R, Jorgensen SJ, Nielsen JS, Klerke A, Mogensen K, Larsens KE, Reimer E, Holm J, Ottesen S. EDTA treatment of intermittent claudication-a double-blind, placebo-controlled study. *J Internal Med* 1992; 231:261-267.
- ³⁷Van Rij AM, Solomon C, Packer SGK, Hopkins WG. Chelation Therapy for Intermittent Claudication: A Double-blind, randomized, controlled trial. *Circulation* 1994; 90(3); 1194-1199.
- ³⁸Knudtson ML, Wyse DG, Galbraith PD, Brant R, et al. Chelation therapy for ischemic heart disease: A randomized controlled trial. *JAMA* 2002;287:481-486.
- ³⁹Holland JF, Danielson E, and Sahagian-Edwards A. Use of ethylene diamine tetra acetic acid in hypercalcemic patients. *Proc Soc Exper Biol Med*. 1953;84:359-364.
- ⁴⁰Doolan PD, Schwartz SL, Hayes JR, Mullen JC, Cummings NB. An evaluation of the nephrotoxicity of ethylenediaminetetraacetate and diethylenetriaminepentaacetate in the rat. *Toxicol Appl Pharmacol* 1967;10:481-500.
- ⁴¹Foreman H, Finnegan C, Lushbaugh CC. Nephrotoxic hazard from uncontrolled edathamil calcium-disodium therapy. *JAMA* 1956;160:1042-1046.
- ⁴²Oliver LD, Mehta R, Sarles HE. Acute renal failure following administration of ethylenediamine-tetraacetic acid (EDTA). *Tex Med*. 1984;80:40-42.
- ⁴³Meltzer LE, Kitchell JR, and Palmon Jr F. The long term use, side effects, and toxicity of disodium ethylenediamine tetraacetic acid (EDTA). *Amer J Med Sci*. 1961;242:51-57.
- ⁴⁴McDonagh EW, Rudolph CJ, Cheraskin E. The effect of EDTA chelation therapy plus supportive multivitamin-trace mineral supplementation upon renal function: A study in serum creatinine. *J Holistic Med*. 1982;4:146-151.
- ⁴⁵Knudston M, Wyse, et al. PATCH Database: Personal Communication. Nov 20, 2002.

⁴⁶Rudolph CJ, McDonagh EW, Wussow DG. The effect of intravenous disodium ethylenediaminetetraacetic acid (EDTA) upon bone density levels. Edited by EM Cranton. In A Textbook on EDTA Chelation Therapy. Charlottesville, VA: Hampton Roads Publishing, 2001;259-264.

⁴⁷Meltzer LE, Rutman J, George P, Rutman R, and Kitchell JR. The urinary excretion pattern of trace metals in diabetes mellitus. Amer J Med Sci. 1962;244:282-288.

⁴⁸Kindness G, Frackelton JP. Effect of ethylene diamine tetraacetic acid (EDTA) on platelet aggregation in human blood. J Advancement Med 1989;2:519-530.

⁴⁹Soffer A, Toribara T, Sayman A. Myocardial responses to chelation. Brit Heart J. 1961;23:690-694.

⁵⁰Centers for Disease Control Advance Data in Vital Health Statistics, number 343, May 27, 2004 available at <http://www.cdc.gov/nchs/data/ad/ad343.pdf>.