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# Ethylenediaminetetraacetic acid : a promising therapeutic agent

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# ETHYLENEDIAMINETETRAACETIC ACID: A Promising Therapeutic Agent

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## A Promising Therapeutic Agent

INTRODUCTION:

During World War II, a relatively new principle was firmly introduced into medical circles; the name applied to this principle was chelation from the Greek chele, meaning claw (1). British-Anti-Lewisi (BAL) was one of the first chelating agents to receive attention and perhaps the first to be widely studied and to arouse enthusiasm for this new principle. Enders (2) and later, German workers headed by Schwartzenbach and Pfeiffer (3), while studying compounds built upon a basic structure of ethylene diamine, prepared the most potent chelator which is presently available, ethylenediaminetetraacetic acid or EDTA.* In recent years, interest in this subject has been mounting rapidly, not only because of its application to thera-peusis, but also because of its potential value in the under-standing of physiological echanisms which have been obscure.

In this thesis, I have made no attempt to completely re-view the literature, which is voluminous, but rather I have

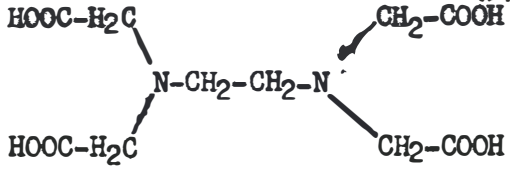
*Ethylenediami etetraacetic acid or one of its salts is also known as A, Edathamil, Endrate, Sequestrene, $SNA_2$, Versene, Versenate, Ethylene bis-iminodiacetic acid, Calsol, Ethylenedinitrilotetraacetic acid, Chelaton, Trilon B, Complexone III, Inminol D, Nervanaid, Nullapon, Idranal III, and Titra Ver; in most cases, I will hereafter refer to the drug as ED A with designation of its appropriate salt by inserting disodium, calcium-disodium, lead, magnesium, or their chemical symbols before the abbreviation.

tried to present a document which would acquaint the reader with the wide variety of applications of the principle of chelation as exemplified by EDTA, thereby serving as an introduction to or a basis for the understanding of a principle that seems certain to receive wide future attention. While I intend to mention all the uses for EDTA which I have encountered in my search of the literature and to explain the theoretical mechanism of effectiveness in each case, my primary interest centers around its application as a treatment of atherosclerosis; therefore, this particular aspect will be discussed in greater detail.

CHEMISTRY & PHARMACOLOGY OF EDTA:

The acid form of EDTA may be prepared by the carboxymethylation of ethylene diamine (4). A variety of preparations in salt form are available in addition to the acid. The chemical structures and properties of the most important are as follows:

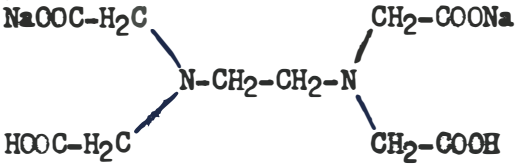
a) Ethylenediaminetetraacetic acid. This is a white, crystalline solid with a molecular weight of 292.1; it is only slightly soluble in water and is a tetra-basic acid (5).




(5)

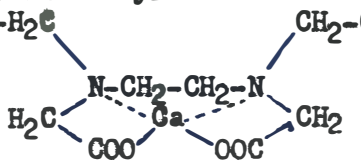
b) Disodium Ethylenediaminetetraacetate ($Na_2H_2EDTA$). This is a white, crystalline compound occurring with two molecules of water of hydration. The molecular weight is 372.10. It is moderately soluble in water, and a 0.1 M solution has a pH of

about 5 (5).



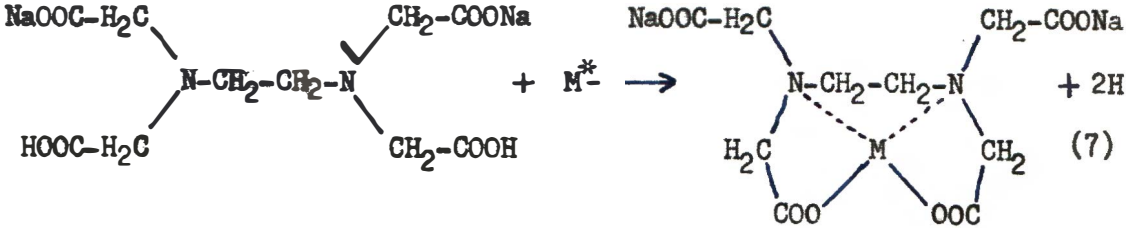
(2)

c) Calcium-disodium Ethylenediaminetetraacetate ($CaNa_2EDTA$).



(6)

Basic to a discussion of EDTA is an understanding of chelation as a pharmacological action. While this is an extremely complex subject, it may be fathomed in an elementary, but workable, fashion. Simply stated, a chelating agent has the ability to de-ionize various metals by entrapping them within a ring structure formed by sharing of an electron pair between the metal and the chelate (ligand), thereby forming a complex. In other words, the chelated metal is firmly bound within a ring structure, and for all practical purposes, it is completely unavailable for utilization by biological systems (1,7). This stable, soluble, metal complex is then easily excreted from the body. This process is analogous to that of ion-exchange resins, and it may be diagramatically represented as follows:



(7)

*M stands for metal ion and may be represented by any one of a number of cations including calcium, magnesium, lead, etc.

When one realizes that oxygen and nitrogen are two of the most common electron donating or electron sharing atoms when contained in a ligand molecule (EDTA has two of each), the potency of EDTA becomes more readily apparent (8).

As intimated by the general designation of metal ion (M) in the above scheme, EDTA is capable of binding a wide variety of different ions. The affinity of EDTA for different important cations is listed below from Welcher (5). In vivo, the element which will be chelated is dependent both upon abundance and relative attraction.

Formation Constants of Metal EDTA Complexes

| Cation | Log K | Cation | Log K |
|---|---|---|---|
| Iron III | 25.1 | Calcium | 10.96 |
| Mercury | 21.80 | Calcium | 10.70 |
| Copper | 18.80 | Hydrogen | 10.22 |
| Lead | 18.04 | Magnesium | 8.69 |
| Zinc | 16.50 | Strontium | 8.63 |
| Cobalt | 16.30 | Barium | 7.76 |
| Iron II | 14.2 | Lithium | 2.79 |
| Manganese | 14.04 & 13.79 | Sodium | 1.66 |

EDTA shows greater affinity for the elements in the table as the log K values rise. Thus, hydrogen may be replaced by calcium, calcium by lead, lead by mercury, etc. It follows from this that the higher the K value, the more stable the complex. Presently, the most widely held concept is that the action of EDTA on biological systems is obtained simply through its sequestration of various cations; however, this does not bar a direct pharmacological action of EDTA, mediated in some other fashion, as a possibility.

The pathway taken by CaEDTA once introduced into the human body was studied by Foreman and Trujillo (9) employing radio-isotope tracer technics. They reported that this agent is rapidly absorbed after parenteral administration with extremely rapid mixing with almost all of the body water, with the exception that it passes quite slowly into the cerebrospinal fluid and it does not enter red blood cells. Slightly more than 95% of the dose is excreted by the kidney, unchanged except for an infinitesmal amount which is metabolized, within 24 hours after injection. Indeed, one-half of the dose can be recovered in the urine one hour after intravenous and two and one-half hours after intramuscular administration. Unfortunately, no more than 5% of a dose is absorbed from the gastro-intestinal tract, and likewise, practically no absorption through the skin occurs, although Popovici et al (10) reported a gradual decrease in serum calcium to 8 milligrams per cent after four daily applications of a 5% water-soluble ointment.

Toxicity:

Although this drug was originally thought to be almost completely non-toxic, subsequent clinical and experimental animal trials have failed to substantiate this belief. It has been realized for some time that the sodium salt, if infused rapidly enough, will produce hypocalcemia of sufficient severity to lead to tetany and eventually to death, if untreated. The work of Bauer et al (11) who tested CaEDTA and PbEDTA suggests

that there is a toxic effect other than that of hypocalcemia. They reported $LD_{50}$'s in mice, rats, and rabbits as being in excess of 4.5, 7, and 6 grams per kilogram respectively after intraperitoneal injection of the calcium salt; intravenous studies in rabbits were reported as below 4 grams per kilogram, however, with no death at levels of 2 grams per kilogram. Dogs given doses of 150, 250, and 500 milligrams per kilogram twice daily showed time-50% mortalities ($LT_{50}$) of 40(27-59), 18.5(12-28), and 13(12-20) days respectively. These doses all exceed recommended human dosage schedules by a substantial amount indicating a rather wide margin of safety.

Reports of clinical toxicity are rather sparse in the literature. Clarke et al (12) and Boyle et al (13) have treated a large number of patients on the following schedule: 5 grams NaEDTA intravenously in 500 cc of 5% glucose and water daily 5 days a week for 2-3 weeks.* This was followed by one to two weeks rest and then resumed, with the total dosage usually between 150 and 300 grams. They have reported that mild gastrointestinal symptoms were commonly noted, but usually were controlled without the interruption of therapy. Stomatitis and scrotal pruritis also occurred occasionally, but usually responded to 25-75 milligrams of oral pyridoxine per day without discontinuation of treatment. A dull ache at the injection site is commonly complained of, but this is of no great moment. Clarke (14) states that in 350 cases treated with NaEDTA, he has

*Currently recommended dosage is 50 milligrams per kilogram per day with the same schedule as above.

encountered no serious toxic effects and he regards the drug as quite innocuous.

Perry and Schroeder (15) also have observed dermatological complications in a single case treated with calcium disodium EDTA in relatively small doses. Cessation of therapy resulted in rapid clearing of complaints. They attributed this to an observed six-fold elevation of urinary zinc excretion.

More serious consequences are reported by Dudley et al (16) in the treatment of two cases of hypercalcemia (one due to osteolytic bone metastases from cancer of the breast and the other from hypervitaminosis D) with intravenous disodium EDTA. In these patients, the daily dosage ranged from 3 to 9 times that which is presently recommended, and treatment was given continuously for 9 and 7 days. Both ended fatally, one with severe hemorrhagic complications and the other due to less clear cut factors, although a rising NPN was noted. Post-mortem findings were similar in the two cases and consisted of hemorrhage, renal damage, and large oval cells with intracytoplasmic eosinophilic granules throughout the reticulo-endothelial system.

In the patient who had shown ante-mortem hemorrhagic manifestations, no post-mortem clotting occurred. It is, perhaps, surprising that recalcification of the blood still produced no coagulation although the addition of prothrombin did. Mixture

of equal parts of the patient's blood with blood having normal prothrombin activity (100%) resulted in 50% prothrombin activity. Prothrombin times measured during treatment in the second patient were found to have diminished to 60 to 70% of normal. Wishinsky et al (17) noted a decrease to 35 to 40% of normal, despite the use of vitamin K, in a patient with hemochromatosis treated with safe doses of $CaNa_2EDTA$. Zucker (18) states that after optimal recalcification, EDTA-plasma clotting and one-stage prothrombin times are significantly longer than that of oxalated plasma, and suggests that this may be due to an interference by EDTA with some ion, possibly magnesium, which has an effect on Factor V (Labile Factor or Ac-Globulin). Leikin and Bessman (19) believe that the prolongation of the one-stage prothrombin time is due to destruction or inhibition of the production of Factor V, but on the basis of their studies, feel that either manganese, zinc, or nickel is the involved cation. It is interesting that Popovici et al (20), in treating hypertensive patients with Mg-EDTA, noted a transient depression in platelet counts with return to normal levels in 24 hours.

The renal damage mentioned above was characterized microscopically by loss of epithelial cells from the proximal and vacuolization of the lining cells in the distal convoluted tubules. In addition, the tubules contained an eosinophilic precipitate. Foreman et al (21) were stimulated to investigate this problem with experiments in rats when a patient under

treatment with 2 1/2 grams of $CaNa_2EDTA$ in 250 cc of saline intravenously twice daily for 12 consecutive days for plutonium poisoning, developed a toxic nephrosis. (It should be noted that the patient recovered with cessation of therapy). They found that in rats the characteristic renal lesion is severe hydropic degeneration of the proximal convoluted tubules and state that it is completely reversible if treatment is discontinued, suggesting close monitoring of the urinalysis during EDTA administration and reluctance to using the drug at all in the presence of acute or chronic renal disease. They theorize that this adverse effect may be due to osmotic factors, as in sucrose nephrosis, which result in the "imbibition of water by the cell" (21); however, Dudley's group points out that the histological appearance of the vacuoles differs in the two conditions. Both the dosage and duration of therapy appear to bear influence in the production of this toxic manifestation.

USES OF EDTA: In Poisonings.

Historically, interest was first directed toward the use of EDTA in lead poisoning, and there is an abundance of literature to substantiate its effectiveness in this disorder (22-31). Examination of the formation constant chart (see page 4) readily reveals a relatively great affinity of EDTA for lead, and this is the explanation for its efficacy in this disease. The reaction seems to proceed in this fashion: $CaEDTA + Pb^{++} \longrightarrow PbEDTA + Ca^{++}$ (22). As might be expected because of the poor absorption of EDTA after

oral administration, this route is relatively ineffective, although Rieders (23) noted a shift from predominantly fecal to mostly urinary excretion of lead in rabbits treated with oral EDTA, and Bradley and Powell (24) suggested its usefulness orally in chronic lead poisoning. EDTA very effectively increases the urinary excretion of lead by either the intravenous (25) or subcutaneous route (26). The latter is particularly useful in infants or small children in whom frequent intravenous infusions are impractical. Bessman et al (25) recommend 1.5 grams of calcium-disodium EDTA subcutaneously or intravenously, suitably diluted, for 5 days, followed by 3 day's interruption of therapy to allow equilibration of bone and soft tissue lead, with subsequent repetition of the cycle until satisfactory clinical improvement obtains. Although EDTA does not enter red blood cells, it effects the release of their lead, apparently through disruption of the equilibrium which exists between RBC and plasma lead (27). It also enhances the loss of lead from the liver (27a). It is important to note that the chelated lead is exceptionally stable and that even when blood levels, which are sufficiently in excess of those which would normally cause symptoms of plumbism, are obtained during treatment, no exacerbation of symptoms occurs (28). This is a striking advantage over a number of other attempted treatments. In a small series, Bradley and Baumgartner (29) have found EDTA to be superior to BAL in the treatment of lead poisoning, with regard to subsequent mental development.

Several animal experiments directed toward assessing the value of EDTA in the treatment of poisoning with radioactive materials have been reported (32-34). These studies indicate that some benefit may be obtained if treatment is rapidly instituted, however, results have been disappointing.

Experimentation with mice has demonstrated that EDTA protects against the alpha toxin (lecithinase) of Clostridium Perfringens (35). This enzyme, which appears to be the important lethal factor in gas gangrene infections, is activated by calcium, and presumably, it is through chelation of this cation that EDTA has its antidotal effect. The authors also suggest that zinc, cobalt, and manganese may have the ability to activate lecithinase.

In Radiology.

A most fascinating sidelight of the observation that lead chelated by EDTA is firmly bound, stable, and rapidly excreted by the kidneys (based on observations in cases of lead poisoning treated with EDTA) led to the theorization that this complex might be employed as a contrast medium by radiologists. Sapeika (36) utilized this substance in various preparations for a) intravenous urography, b) esophograms, and c) visualization of the paranasal sinuses, finding it to provide excellent contrast with very little evidence of toxicity. Shapiro (37) noted that intravenous administration in animals resulted in highly satisfactory opacification

of not only the renal pelves, ureters, and bladder, but also the renal parenchyma itself, 15 minutes after injection. He was also able to visualize the liver, spleen, and cardiovascular system by this route, and orally, the upper gastro-intestinal tract. Unfortunately, he was able to corroborate the toxicity experiments of Bauer et al (11) and concluded that presently available lead chelates were probably contraindicated in contrast Roentgenology because of the danger of toxic side effects; however, development of more stable complexes may eventually obviate this problem.

In the Laboratory.

Because of the ability to chelate calcium, the sodium salt of EDTA has been found to be efficacious as a demineralizing agent in the preparation of bones and teeth for histological examination (38,39). There is no appreciable loss of structural detail or staining properties, as is prone to occur with some solvents.

As the reader will recall from the discussion of toxicity of EDTA, there is a definite prolongation of prothrombin time, in vivo. Dyckerhoff et al, as cited by Proescher (40), first investigated the in vitro effect on blood coagulation and stated its effect to be due to chelation of calcium ion. Proescher (40) and Wittgenstein (41) found $Na_2EDTA$ to be an excellent anticoagulant for blood banking and routine hematological work, respectively, citing its potency, low rate of damage to cellular elements, and lack of adverse effects, when infused, as its advantages. Klapheke

and Rubin (42) tested $Na_2EDTA$ against other anticoagulants in routine laboratory procedures, e. g. blood sugar, BUN, NPN, creatinine, TSP and A/G ratio, carbon dioxide combining power, uric acid, and various hematological tests, and obtained essentially identical results in all determinations with the exception of the NPN which was elevated by 3 to 5 milligrams per cent in the EDTA specimens.

A further laboratory use of EDTA is in determinations of cerebro-spinal fluid calcium and magnesium as reported by Bair et al (43). Welcher (5) has devoted an entire book to the analytical uses of EDTA.

In Hemochromatosis.

Wishinsky et al (17) attempted to enhance the excretion of iron in a patient with hemochromatosis employing $CaNa_2EDTA$ intravenously. Results were rather disappointing in that a total of 8.2 grams of the chelating agent resulted in an increased excretion of only about 10.2 milligrams of iron. Phlebotomy of 100 milliliters of blood removes about 50 milligrams of iron.

In Porphyria.

Chelation therapy employing British-Anti-Lewisite alone, a combination of BAL and EDTA, and EDTA alone in patients with various types of porphyria has been reported by Peters et al (44,45). Favorable results were obtained in 31 of 37 cases with no adverse effects noted. Complete recovery from tetraplegia due to porphyric motor neuropathy was reported. The authors credit maximal

nursing care; tracheotomy; attention to electrolyte balance; avoidance of barbiturates, sunbathing, steroids, and heavy metals; and physical therapy as being essential to their success. Regarding the rationale for chelation therapy, the authors state as follows:

> "Symptoms of porphyria are thought to be due to zinc, copper, or other cation block of several metallo-enzyme systems. Exhaustion of certain portions of the body's natural chelation defenses and subsequent depletion of porphyrins for purposes of chelation may likewise impoverish the metabolism of the body and affect the maintenance of myelin and the cytochrome systems that interrelate to a 'porphyrin pool.' Artificial chelation is felt to replenish the depleted reserves of the body, thus restoring normal chelation." (44)

Woods et al (46) have reported a single case of cutaneous porphyria successfully treated with <u>oral</u> calcium-disodium EDTA over an extended period of time (total dose was 185.5 grams).

<u>In Hypertension.</u>

Popovici et al (20) tested the efficacy of the magnesium chelate of EDTA in essential hypertension based on the known hypotensive effect of magnesium. They reasoned that since the depressive action of magnesium on neuromuscular transmission and central nervous system activity has been observed to be antagonized by excessive calcium ions, an agent which would release magnesium and at the same time bind serum calcium would exert maximum effect. In eight cases of hypertension treated with a total dose of from 10 to 40 milligrams of MgEDTA intravenously, lowering of both systolic and diastolic pressures was noted, usually accompanied by bradycardia. The maximum decreases in pressures were 80 mm Hg systolic and 40 mm Hg diastolic. The depres-

sion gradually returned to pre-treatment levels in about six week's time. Serum calcium levels were transiently lowered with return to normal within 24 hours.

In Hypercalcemia.

The observation that the sodium salt of EDTA is capable of forming chelates with calcium ions with rapid excretion from the body (10) has led to the investigation of its use in a variety of disease processes. Bellin and Laszlo (47) demonstrated this effect using radio-isotopic technics finding that the excretion of $Ca^{45}$ and calcium increased ten-fold after the infusion of disodium EDTA. On the basis that 1 gram of this agent is theoretically capable of complexing and excreting 108 milligrams of calcium, it is 72% efficient in the first 24 hours after infusion, gauged by the measurement of urinary calcium (48).

Surprisingly enough, despite the fact that the sodium salt of EDTA is able to produce hypocalcemia leading to death if given rapidly enough intravenously (10), it has been found to be only transiently effective in reducing chronically elevated serum calcium levels, e. g. in patients with multiple myeloma, hypervitaminosis D, osteolytic bone metastases from carcinoma, etc. (16, 49). Even with prolonged therapy with extremely high doses, temporary fall with rebound to unphysiologic levels occurs although the resultant level may be somewhat lower than that prior to therapy. No readily apparent explanation for this phenomenon is available; however, the repetitive lowering of serum calcium

may evoke a hyperactivity on the part of the parathyroid gland.

In Digitalis Toxicity.

The more purified preparations accentuate the problem of digitalis toxicity because of the decreased incidence of gastrointestinal side effects. The fact that calcium ion is synergistic with digitalis has been recognized for some time, and this has been utilized in the application of $Na_2EDTA$ to digitalis induced arrhythmias by Gubner and Kallman (50). They reported on the treatment of three such cases who were in heart failure and given 600 milligrams $Na_2EDTA$ diluted in 250 cc of 5% glucose and water in a single injection. Marked clinical and electrocardiographic improvement was noted which persisted after return of serum calcium levels to normal. Interestingly enough, the same preparation transiently controlled twenty-nine cases of non-digitalis arrhythmias.

In Corneal Opacities.

Grant (51) reported the successful dissolution of calcific corneal opacities in six cases by irrigation for fifteen minutes with a sterile, neutral, 0.01M solution of EDTA under local anesthesia. Where the opacity was covered by epithelium, a denuding procedure was performed. At this solution strength, there was no appreciable tissue irritation, yet there was sufficient potency to make the treatment practicable. The author recommends that the irrigation be carried out within the first 24 hours in lime burn cases.

In Scleroderma.

Klein and Harris (52) in a case of scleroderma, sclerodactylia, and calcinosis, and Rukavina et al (53) in three cases of non-calcific scleroderma, employed chelation therapy in the form of the sodium salt of EDTA by the intravenous route over several week's time. Marked clinical improvement was noted in all four of these cases with changes such as decreased induration of the skin with loss of the porcelain-white appearance and return of the ability to wrinkle the forehead, increased range of joint motion, increased tolerance to cold, healing of fingertip ulcers, and increased chest expansion being observed. The subjective improvement was corroborated by pre- and posttreatment x-ray evidence of diminution of articular and cutaneous metastatic calcium deposits in Klein and Harris' case. These workers felt that benefit was derived primarily through the chelation of calcium, while Rukavina's group noted abnormal tryptophan metabolism (53), in their three cases, which was improved (as gauged by the observation of decreased urinary excretion of kynurenine, hydroxykynurenine, kynurenic acid, and N-alpha-acetyl-kynurenine which were formerly noted to be excessive) by the administration of EDTA, as well as pyridoxine and nicotinamide. Despite this finding, they suggest that improvement may be mediated through alteration in calcium metabolism or action on an obscure enzyme system.

In Urinary Calculi.

Chelation with disodium EDTA has also been applied to a problem which prior to the days of anesthesia and antiseptic surgery was, without treatment, inexorably attended by severe pain and eventual death and, with the best treatment of the time, destined to be fatal in more than 50% of cases. I am referring to urinary tract stones. Development of a non-operative treatment technic is desirable because of the likelihood of recurrence in this disease. Gehres and Raymond (54) and Abeshouse and Weinberg (2) have approached this problem by in vitro and in vivo dissolution experiments. Their in vitro tests indicate that $Na_2EDTA$ is effective against all types of stones tested although urate calculi are the least suseptible. In vivo tests with a 10% solution of versene were attended by rather severe imflamatory reaction, however, less concentrated solutions, while only slightly less potent, caused much less irritation. Preliminary clinical trials were partially or wholly successful in 4 of 7 patients with renal or bladder calculi (54).

Clarke et al (55) report the case of a 51-year-old man with x-ray evidence of nephrocalcinosis plus dimunition in hearing for 15 years. He was treated with 575 grams of $Na_2EDTA$ ( a total of 100 infusions given over an extended period of time) suitably diluted in 5% glucose and water for intravenous injection. In addition to 50-65% clearing of the renal stones by x-ray after treatment, the watch test for his left ear improved from 3 inches

to 28 inches. The authors felt that improvement was obtained because the serum calcium, which was complexed by the EDTA, was replenished from areas of metastatic or pathological calcification more readily than from bone. It was this latter theorization which led Clarke, Boyle et al to the fascinating investigation of EDTA as a treatment for atherosclerosis, which I will discuss below.

In Atherosclerosis.

While it is outside the scope of this paper to review the many theories regarding pathogenesis of atherosclerosis, certain statements are in order.

The use of EDTA in the treatment of atherosclerosis is predicated on the belief that the calcium which is seen in plaques is not deposited there secondarily, but rather, that calcium, magnesium and perhaps other cations are active in the initial stages of atheroma formation. If this be true, it is possible that removing these minerals from the plaques may result in degeneration of these pathological formations. Lansing (56) in studying human aortas at various ages, has noted progressive splitting and fraying of the elastic fibers of the internal elastica beginning at about the age of 20. These areas have a profound and demonstrable affinity for calcium, and the content of this element becomes progressively greater with aging even without gross evidence of atheromata. He states that while he has noted calcification without the presence of plaques, he has never seen plaques without calcification. He concludes that while these observations do not

refute the importance of altered cholesterol metabolism, the process appears to be a dual one in which aging of the arterial wall provides a suitable substrate for the accumulation of cholesterol.

Uhl et al (57) investigated the effects of EDTA in rabbits on a high cholesterol diet noting definite changes as compared to controls. Grossly fewer vascular atheromata were noted in the treated animals despite significant elevation of blood cholesterol levels. On the contrary, hepatic cholesterol was found to be markedly higher in the control animals as compared to those on EDTA. Calcium and magnesium levels tended to parallel those of the cholesterol in blood and liver--that is, where cholesterol was found to be high, calcium and magnesium were also elevated, suggesting a metabolic interrelationship. Animals fed high cholesterol diets and subsequently treated with EDTA showed normal hepatic cholesterol indicating the ability of the drug to promote the removal of deposits. Curran (58) found that EDTA increased the hepatic cholesterol synthesis in rats, theorizing that its action may be mediated through chelation of vanadium, thereby removing this known inhibitory factor. The above facts were found to hold true for both the calcium and sodium salts of EDTA.

Interestingly enough, Perry and Schroeder (15,59) found that in humans, blood cholesterol concentration, in contradistinction to rabbits, was depressed an average of 75 milligrams per cent

after a mean, total, intravenous dose of 18 grams of Calcium-Disodium EDTA. They suggested, vaguely, that this result is obtained through an effect on an enzymatic process. Schroeder (60) has combined oral chelation therapy with diet and pyridoxine in a method for reducing plasma cholesterol with fairly good result.

Clarke et al (12) in 1956 published an article reporting on the results of chelation therapy with disodium EDTA on 20 cases of angina pectoris. Patients were selected who had typical anginal type chest pain which had increased in frequency and severity or who had historical and electrocardiographic evidence of old myocardial infarctions. Several suffered from intermittent claudication. Improvement was based on measured physical activity rather than the patients' subjective evaluation. The drug was administered in 5 gram doses diluted with 500 cc of 5% glucose and water or normal saline intravenously over $2\frac{1}{2}$ to 4 hours time. This was usually given five days per week until 10 to 15 infusions had been performed. Cessation of therapy for 1 to 2 weeks was then followed by a second series of injections. The number of infusions ranged from 15 to 60. Uniformly good results were obtained with rather striking improvement in exercise tolerance retained in many cases for 12 to 18 months without further treatment. Electrocardiograms reverted to normal in about 50% of patients who had not had previous infarctions, but who had shown evidence of myocardial damage in pretreatment tracings taken at rest. A single death occurred thought due to embolization of a degenerating atheromatous plaque. Although no controls were run, a placebo effect was dis-

counted because little if any clinical progress was noted usually until 20 or more infusions had been given.

Serum stability studies which were performed led the authors to conclude that surface tension plays an important role in the production of atherosclerosis. They found decreased surface tension with increased age which was elevated for several weeks after the infusion of disodium EDTA. This may have been mediated through chelation of polyvalent positive ions, which were known to decrease serum stability.

Boyle et al (13) have summed up the application of chelation to this important disease of the aged in the following logical manner:

> "Plaques which are laid down in and on the vascular wall possess a larger proportion of calcium and magnesium than is contained in adjacent tissue. It is not inconceivable that such plaques result from chelation, or complexation of these metals by protein and connective tissue components such as mucopolysaccharides. Introduction into the blood stream of a chelating agent which would strongly bind calcium ion would in effect reduce the available calcium. If such an agent is given parenterally not only would the calcium ion be bound, but also a competition for the bound calcium of the albumin complex would result. The circulating serum now is unsaturated with respect to calcium. In this situation the blood demands calcium from other sources to satisfy the needs of albumin and also have enough calcium ion for physiological and chemical processes to continue. That they do continue when enough ethylenediaminetetraacetic acid is injected into a rabbit to more than chelate all the calcium, both bound and free, in the extracellular fluid demonstrates that the mobilization of calcium ion is extremely rapid. The question arises as to what mechanism is employed to equilibrate this unbalanced system. It is not unreasonable to speculate that the parathyroids responsible for serum calcium level play a part in this equilibration."

> "In effect a secondary hyperparathyroidism is produced which results in quickly mobilizing calcium from numerous body depots. It would appear that the amount of calcium mobilized from any one depot would be related inversely to the binding influence of the depot. In other words the more tightly bound calcium would yield less ion in response to the demand. It seems reasonable that demands for calcium would be made upon atheromatous plaques in consequence of which inhibition of plaque growth and even dissolution of plaque substance may occur. It is apparent that EDTA does not directly attack calcium depots, metastatic or otherwise. Its effect is more likely to be indirect and mediated through serum with induced low calcium ion concentration as well as physiological stimulation of parathyroid activity."

Clarke and his group have continued their study in a much larger series of cases and he states that further use of EDTA in the treatment of angina pectoris has continued to impress him with its real value in this disease (14). As a final note, although one might theorize that the in vivo anti-coagulant effect which has been noted (see section on toxicity) must be assessed as a possible beneficial action accounting for some, if not all, of the satisfactory clinical response, this seems unlikely due to the length of time after treatment which improvement has persisted.

SUMMARY:

The preparation of ethylenediaminetetraacetic acid and its various salts has provided a group of potent chelating agents for medical use. Their chemical structures have been herein reviewed. The effect of these drugs appears to occur through the sequestration or complexation, followed by excretion, of certain biologically active cations in a rather predictable

fashion, thereby rendering them unavailable for their usual physiological functions. EDTA is rapidly absorbed from parenteral sites and quickly mixes with almost all of the body water. It is almost entirely excreted, with its inactivated cation, by the kidney within the first twenty-four hours. Mild toxicity in the form of dull ache at the injection site, mild gastro-intestinal upset, stomatitis, and scrotal pruritis occurs not infrequently with prolonged administration of reasonable doses; however, these may often be controlled without cessation of therapy. Too rapid infusion of the sodium salt may result in hypocalcemia leading to death, but this is easily avoided. Much more serious untoward reactions consisting of toxic nephrosis and hemorrhagic complications have been recorded when prolonged therapy with extremely high doses has been employed.

Among the first and most logical uses of EDTA was in the treatment of lead poisoning. It has been found to be an excellent agent in this disease. Much less benefit has been obtained in experimental cases of radioactive contamination. The alpha toxin of Clostridium Perfringens is inactivated by EDTA in vivo.

Attempts to employ the lead chelate (PbEDTA) as a radiographic contrast medium proved to be too toxic for routine use, despite excellent opacification of kidneys, ureters, bladder, gastro-intestinal tract, liver, spleen and cardiovascular system.

Disodium EDTA has been successfully used in the demineralization of bones and teeth for histological examination. It has also

been utilized in the laboratory as an anti-coagulant and in titrations.

Iron excretion in a case of hemochromatosis was not appreciably enhanced.

Prolonged chelation therapy combined with great care in supportive measures gave satisfactory results in 31 of 37 cases of porphyria.

Eight cases of essential hypertension were benefitted for about six weeks after low doses of magnesium EDTA.

Unfortunately disodium EDTA has been only transiently effective in reducing hypercalcemia due to osteolytic bone metastases, multiple myeloma, hypervitaminosis D, etc. However, the calcium binding effect has suggested its usefullness in digitalis toxicity, dissolution of calcific corneal opacities, scleroderma, urinary tract calculi and incident to the latter, otosclerosis. Clinical trials have substantiated the efficacy of disodium EDTA in these conditions.

The chelation of calcium also led researchers to employ EDTA in the treatment of the complications of atherosclerosis. Nineteen cases of severe angina pectoris with or without a history of previous infarctions were reported to show rather marked improvement based on exercise tolerance, electrocardiographic changes, and nitroglycerine requirement. One death occurred. Subsequent unpublished clinical investigation has continued to demonstrate EDTA's beneficial effect in this disease.

CONCLUSION:

Chelation has provided a relatively new medical horizon. EDTA, representing the strongest agent of this type, already has been variously used in the clinical laboratory and has been applied to the treatment of atherosclerosis, scleroderma, digitalis toxicity, lead poisoning, calcific corneal opacities, gas gangrene, porphyria, hypertension, and urinary calculi with some success. Results have been disappointing in other disease entities. Chelates other than those formed by EDTA show promise in other ways, e.g. obviating the gastro-intestinal side effects of iron therapy (61) and delivering radioactive materials to tumor tissue (62).

Unfortunately, history is replete with recordings of "brief candles" which have burned brightly momentarily only to be extinguished by more thorough scientific investigation. Although EDTA, and perhaps numerous other chelating agents, may fall into disrepute under careful scrutiny, chelation itself seems certain to serve a very useful role in the future.

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