**Zeitschrift:** Bulletin der Schweizerischen Akademie der Medizinischen Wissenschaften = Bulletin de l'Académie suisse des sciences médicales = Bollettino dell' Accademia svizzera delle scienze mediche

**Herausgeber:** Schweizerische Akademie der Medizinischen Wissenschaften

**Band:** 13 (1957)

**Heft:** 1-4: Symposium über Arteriosklerose = Symposium sur l'artériosclérose = Symposium on arteriosclerosis

**Artikel:** Studies in human and induced atherosclerosis employing ethylenediaminetetraacetic acid

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**DOI:** https://doi.org/10.5169/seals-307335

## 3. Klinik und Therapie – Clinique et thérapeutique – Clinic and therapy

D.C. 616.13-002.2-08

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# Studies in human and induced Atherosclerosis employing Ethylenediaminetetraacetic acid[1]

By **Albert J. Boyle, J. J. Jasper, Herbert McCormick, Mary Kosai, Daisy McCann,** and **Jesse Goodwin Norman E. Clarke,** and **Robert E. Mosher**

Before discussing the use of salts of ethylenediaminetetraacetic acid (EDTA) in the treatment of coronary artery disease I would like to point out a few of the experimental findings in rabbits with induced atherosclerosis made by our group at Wayne State University, Detroit, Michigan. It also would appear pertinent to discuss at some length the philosophy which led to the use of EDTA in atherosclerosis and suggests its further use in several metabolic disorders.

In our early experimental work (1) it was shown that rabbits (Group B) placed on a high cholesterol diet and injected subcutaneously with 500 mg of sodium EDTA every other day developed serum cholesterol levels that were elevated above that of similarly fed control animals designated Group A (Fig. 1). On sacrifice, all test animals, numbering eleven in each group, presented aortic atherosclerotic plaques. One impressive finding was the marked decrease of liver fat and cholesterol in the injected group (Fig. 2) as revealed by inspection and chemical analysis. Serum electrolyte studies in this series of animals showed a depression in serum calcium levels (Fig. 3) though considerably greater in the injected Group B at the midpoint of the experiment which lasted 111 days[2]. Thereafter, however, Group B serum calcium rose to meet the level noted in Group A. Serum magnesium fell initially in both groups and ultimately returned

[1] Financed by Grant-in-Aid from the Michigan Heart Association and Ciba Pharmaceutical Products, Inc.

[2] From day 48 to 72 no injections of EDTA were given to Group B. During part of this period CaEDTA was fed.

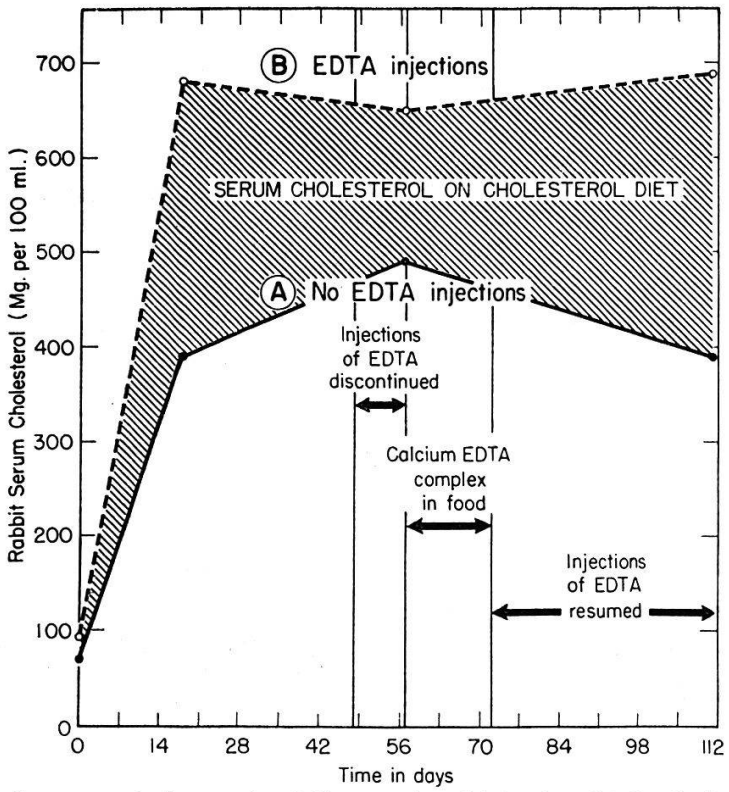



Fig. 1. Values of serum cholesterol of Group A rabbits (on high cholesterol diet) and of Group B rabbits (on high cholesterol diet plus EDTA).

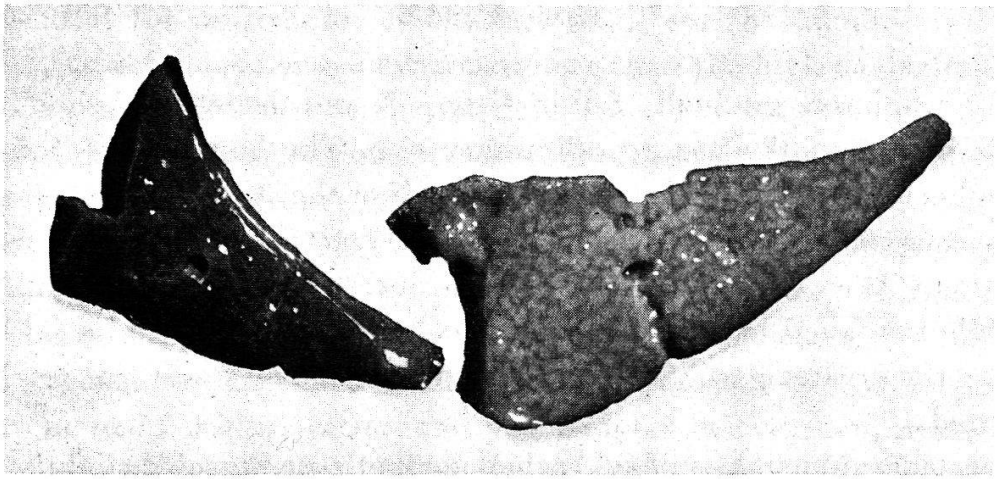


Fig. 2. Left: Normal liver from Group B rabbits on high cholesterol diet plus EDTA. – right: Liver infiltrated with cholesterol from Group A rabbit on high cholesterol diet alone.

to approximately initial values in Group A, but rose to supernormal levels in Group B.

Total serum phosphorus rose during the first half of the study to reach higher levels in Group B than in A. These values were maintained at a plateau during the rest of the study in Group B, but fell off to the initial levels in Group A. The elevation at the midpoint of the study was attributable to increase in acid-soluble phosphorus in both groups, since

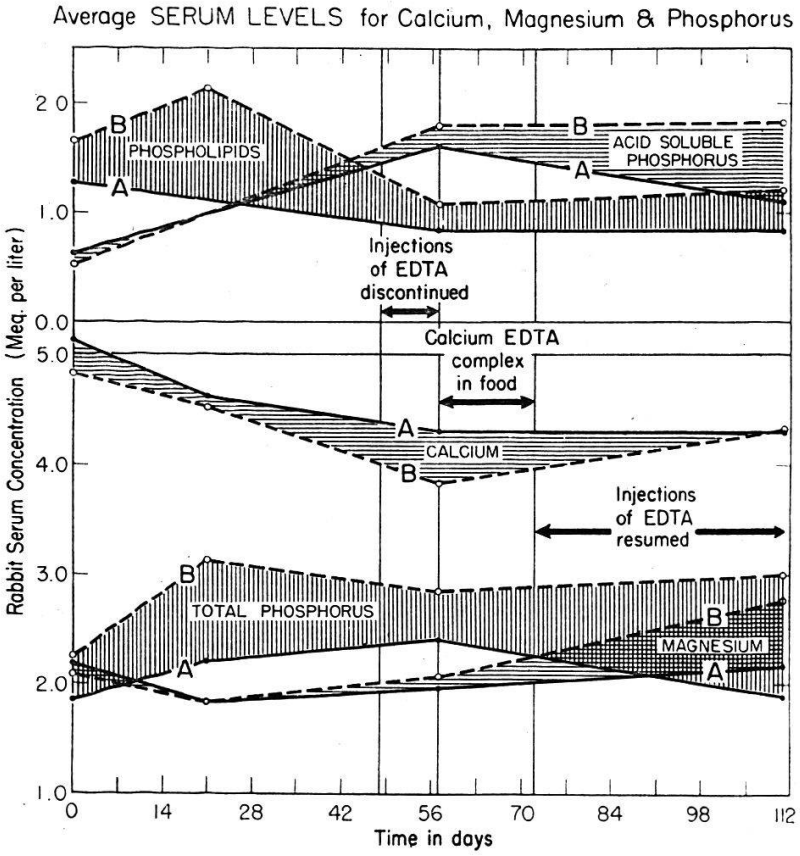



Fig. 3. Average serum levels of calcium, magnesium and phosphorus for rabbits of Group A and B.

lipid phosphorus gradually fell in Group A and decreased below initial levels in Group B after a preliminary rise. The high plateau of total phosphorus in Group B during the last half of the study was attributable to maintained elevation of the acid-soluble fraction, whereas the decline in Group A was referable chiefly to a decrease of this fraction. Lipid phosphorus failed to parallel cholesterol and was below the initial levels during the greater part of the experiment when cholesterol was markedly elevated.

Spectrographic ratios of acid ashed aortic tissue revealed slightly more calcium and magnesium in Group B than in Group A. On the other hand, hepatic calcium and magnesium, as well as cholesterol, were significantly lower in Group B. It would appear that the larger the quantity of cholesterol laid down in these respective tissues, the higher the accompanying concentration of calcium and magnesium.

Another noteworthy postmortem observation in rabbits that had received sodium EDTA was a marked decrease in fat deposits in the abdominal cavity even in the presence of maintained weight or normal gain in weight throughout the experiment. Very striking was the absence of perirenal fat in the injected Group B. The adrenal glands of the rabbits

of Group B were considerably larger. This may have represented a reaction to the irritation from the injections which produced rather severe skin lesions at the site of injection. The average combined weight of the adrenal glands was 0.852 g for the animals of Group B, as compared with 0.619 g for the animals of Group A.

From this study it would seem that sodium EDTA aggravates dietary hypercholesterolemia in the rabbit, but protects against deposition in the liver and seems to remove stored cholesterol from the liver. (The latter has been shown by using rabbits of the Group A type that have been on high cholesterol diets for months. Three weeks of EDTA injections every other day results in a liver essentially normal in cholesterol content as opposed to controls.) The higher serum cholesterol of Group B may merely reflect primary interference with storage of cholesterol in the liver. The known effect of EDTA in complexing calcium and magnesium ions and the parallelism of cholesterol, calcium, and magnesium concentrations in the aorta and liver raise the question of a significant interrelation of cholesterol, calcium, and magnesium metabolism. The removal of trace metals by EDTA has also been considered as a factor in the altered metabolism of cholesterol in this study. The possibility exists, however, that the deposition of calcium and magnesium in tissue is essential for infiltration of cholesterol. If so, the administration of EDTA, under more normal circumstances than the high cholesterol diet provides, may interfere with the deposition not only of calcium and magnesium, but also of cholesterol in the tissues or may promote the removal of all three from preexistent deposits. This suggests possibilities in other diseases involving calcium, particularly the collagen group.

The greater weight of the adrenal glands in Group B raised the question as to whether or not the discrepancies in cholesterol metabolism were referable to differences in activity of the adrenal cortex. To obviate the element of skin irritation and yet complex calcium ion, magnesium EDTA was used in further experimentation. Calcium is bound by EDTA about one hundred times more effectively than magnesium, thus when the magnesium EDTA is introduced it very soon is converted to CaEDTA which is excreted in the urine along with excess magnesium ion. There is little doubt that the irritation from subcutaneous or intramuscular sodium EDTA is due to the removal of magnesium and calcium from tissue. No irritation is noted at the site of injection from calcium or magnesium EDTA and the adrenals are not enlarged.

Rabbits were used to investigate some of the above possibilities and the results are described in a paper now in preparation. Because this discussion must not be too time – consuming only the highlights of more

recent work will be given in outline form. A number of experimental situations are presented with a brief comment on the findings.

1. The effect of the parenteral administration of calcium EDTA in rabbits on a high cholesterol diet.

*Comment:* This situation presents no apparent change in serum cholesterol levels as opposed to controls.

2. The effect of oral administration of calcium gluconate on serum cholesterol levels in rabbits fed a high cholesterol diet.

*Comment:* The oral feeding of calcium gluconate does not alter serum cholesterol levels as opposed to controls.

3. The effect of subcutaneously injected magnesium EDTA in rabbits previously given atherosclerosis and then placed on a normal diet.

*Comment:* In this situation the aortas of the injected animals show fewer plaques on inspection and less cholesterol by chemical analysis. Somewhat more cholesterol is present in the livers and adrenal tissue as compared to controls, but both groups are within normal limits.

4. The effect of subcutaneously injected magnesium EDTA in rabbits fed a high cholesterol diet.

*Comment:* Magnesium EDTA does not appear to cause a significant rise in serum cholesterol levels over controls. It does, however, noticeably prevent aortic plaque formation and reduces the amount (though not as successfully as sodium EDTA) of cholesterol in the liver.

5. The effect of subcutaneous injection of magnesium salt of EDTA in rabbits on a normal diet.

*Comment:* Greatest changes in this experiment are found in serum magnesium and calcium levels. The initial lowering of the serum calcium level approaches normal over a period of 7 weeks, suggesting that parathyroid activity is increased. The injected group shows a slightly higher cholesterol content of liver and adrenal tissue but within normal limits.

During the past few years considerable interest in calcium metabolism has been evinced by researchers in biological studies. *Smith* (2) has demonstrated quite conclusively that normal human serum calcium levels (Table 1) are not as high as formerly supposed. Newer techniques have demonstrated that ionized calcium in the serum represents approximately 50% of the total calcium present as compared to the 20% which has, until recently, been the widely accepted figure (3). *Gutman* has spent considerable time proving that calcium binding in serum is accomplished largely by albumins (4, 5). The complicated story of how calcium is absorbed from the intestine, how it is maintained at fairly constant level in serum, its excretion in the urine is as yet scarcely known. For this discussion it may be stated that vitamin D, phosphorus, phosphatase, parathormone and other substances all play a role in calcium metabolism.

Table 1

Summary of averages, ranges and deviations of metallic constituents of normal plasma (Concentrations expressed in milliequivalents per liter of plasma)

| | Na FL. PH. | Na Spec. | K FL. PH. | K Spec. | Fe Spec. | Mg Spec. | Ca Spec. |
|---|---|---|---|---|---|---|---|
| No. of samples | 70 | 100 | 73 | 103 | 101 | 103 | 86 |
| Average value | 142 | 145 | 4.06 | 4.11 | 0.068 | 1.59 | 4.30 |
| Range of values | 136–158 | 130–159 | 3.61–4.85 | 3.40–4.92 | 0.024–0.195 | 1.01–2.12 | 3.04–5.27 |
| Average deviat. mEq./L. | 1,8 | 5.5 | 0.26 | 0.27 | 0.025 | 0.12 | 0.32 |
| Average deviat., per cent of average | 1.3 | 3.8 | 6.4 | 6.4 | 37.0 | 7.6 | 7.4 |
| Average deviat. between flame photometric and spectrographic data on 70 samples mEq./L. | 5.5 | | 0.25 | | | | |

Something more, perhaps, is understood regarding the part calcium plays in blood clotting. Less is known concerning its profound effect on the nervous system.

The skeleton contains approximately 99% of all body calcium. Only 1.2 g of calcium of the several pounds present in the average human are found in body fluids and most of this is bound by various tissues. Indeed, less than 400 mg is in the circulating plasma and at least 50% of this is bound by albumin.

The above facts lead to considerable speculation and in part form the basis for some of the studies described. Work, earlier on in this laboratory, was directed at a study of the binding properties of serum protein relative to the second ionization potential of metal cations (6). It was found that the higher the ionization potential the greater the binding of protein by the metal. The relationship was demonstrated by measuring the smallest amount of metal ion essential to elicit a turbidity in serum. Mn II, Fe II, Co II, Ni II, Cu II, and Zn II ions were used among others. These ions all have the same charge of plus 2 but possess different field strengths because of variations in size. Their turbidity-producing power was shown to correlate nicely with ion size. That is, the smaller the ion the greater its electric field strength or second ionization potential and the less of it required to produce turbidity in serum. Moreover, it was shown that the successive addition of two of these ions, each in smaller quantity than required to produce turbidity individually, was additive in combined effect, provided the ion of greatest field strength was added

first. This relationship suggests quite clearly why so few divalent ions circulate in blood. Body cells contain most of the polyvalent ions such as phosphorus, calcium and magnesium. Their presence in conjunction with protein yields the solid structures of the body. In their absence the protein of the cell is mobilized and lost to the circulation. In effect a simple way to visualize cellular structure is that it is the product of protein complexation and chelation of polyvalent ions (Fig. 4). It will

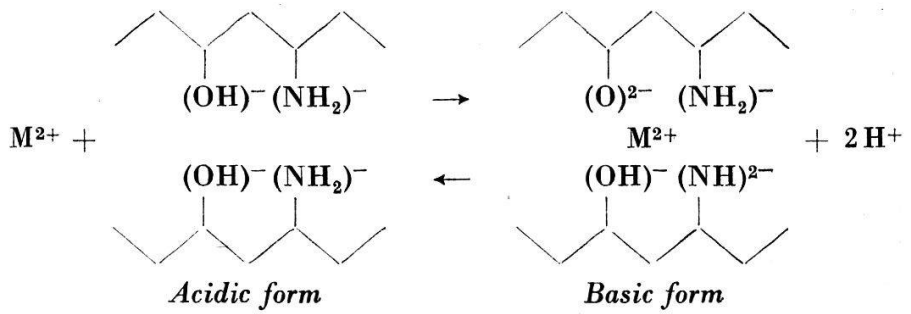



Fig. 4. Equilibrium situation involving protein, metal and hydrogen ion.

be recognized that this is a dynamic situation and that changes in hydrogen ion will shift the elements of the complex. One might show a similar diagram to illustrate the equilibrium between calcium and connective tissue. 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.

It would appear that in part at least the maintenance and growth of atheromatous plaques is brought about by depositions from plasma (7). It is significant that such deposits occur in areas of turbulent flow such as the aorta and the gravity-fed coronary arteries which are compressed and dilated respectively, with each heart beat. It does not appear that cholesterol concentration per se in the plasma is responsible for the formation of plaques but rather its precipitation from an unstable colloidal situation in plasma. Under the influence of normal enzymatic and emulsifying activities of the intestinal juices, dietary fat is hydrolyzed and saponified. Within the mucosa of the intestine it is united with glycerol to form neutral fats or phosphorylated to form phospholipids. Colloidal phospholipids and cholesterol esters then may enter the blood stream by way of the lymphatics or directly through the blood vessels of the intestinal mucosa. The phospholipids are hydrophilic and the cholesterol esters hydrophobic. The proper ratio of cholesterol to phospholipid tends to render the system stable. Absolute amounts appear to be less important.

Undoubtedly the liver has a great deal to do with the colloidal stability of serum. It protects from dietary cholesterol (8) in the human and many animals. On the other hand, the rabbit has much less of this liver efficiency. To this animal cholesterol is a rather rapid poison. It might be reasoned, therefore, that any beneficial effects experienced in the atherosclerotic rabbit as the result of EDTA might prove even more beneficial to the atherosclerotic patient on EDTA.

The colloid stability of serum depends upon the hydration and charge on the protein molecules. One approach to test colloid stability used by our group was a metal precipitation of serum protein. More recently we have come to believe much may be learned about colloid stability through serum surface tension measurements (Fig. 5).

The preliminary experiments with serum indicate that the surface tension decreases with time. Since the surface tension of a pure liquid

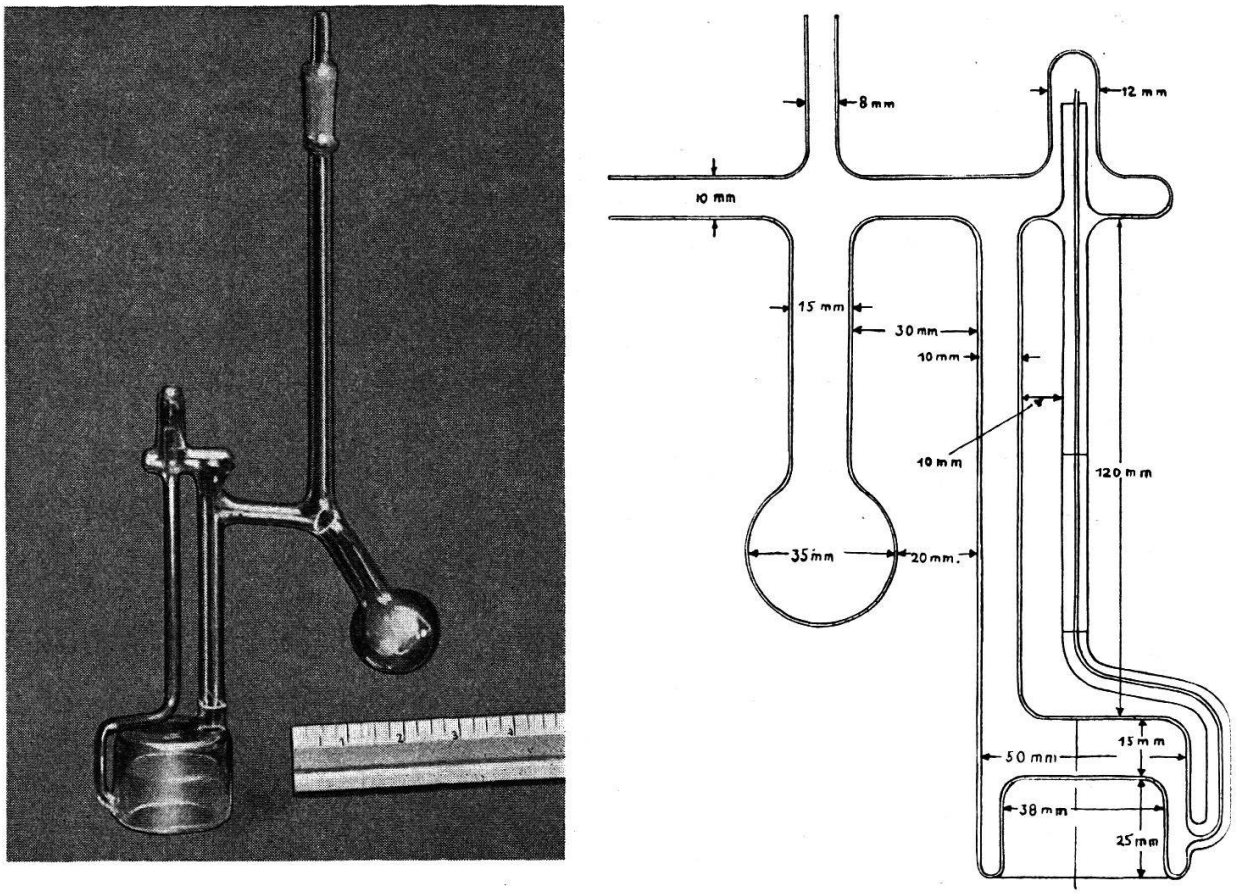



Fig. 5. Apparatus for surface tension measurement of serum. Measurements are made at 37° C. This apparatus is described by *Jasper* and *Herrington:* J. Amer. chem. Soc. **68,** 2142 (1946).

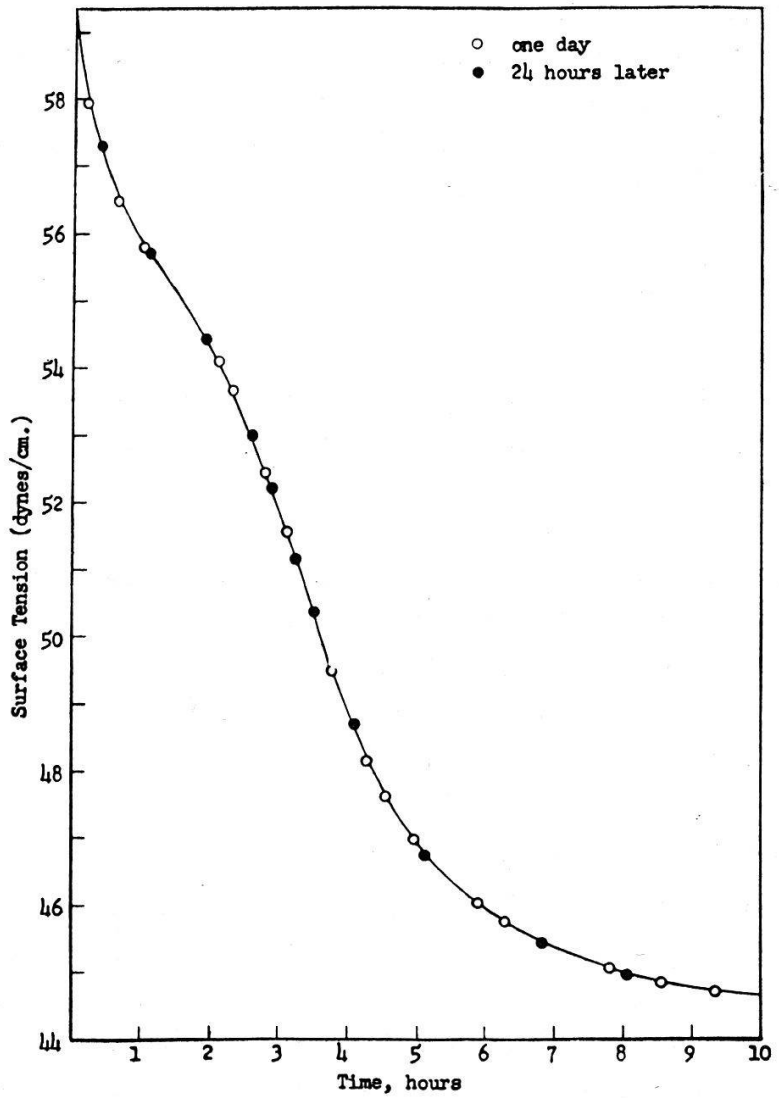
remains constant under isothermal conditions, it is obvious that serum is not pure but holds in colloidal or molecular dispersion components whose surface tensions are greater or less than that of the dispersion medium in question. If the surface tension decreases with time, this indicates the presence of capillary active substances which have migrated to the surface and have been positively adsorbed thereon. On the basis of these well established facts, it seems to us that a most significant pattern of behavior reveals itself in surface tension versus time studies of serum. The shape of such curves clearly indicates that the time derivative of the surface tension varies greatly for different sera, but each approaches the same equilibrium value after about ten hours.

Assuming the colloidal stability of serum protein is dependent upon the hydration and charge on the protein molecule stimulates speculation. A change in the charge carried by the molecules would then alter colloid stability. If the slow rate of surface tension decrease is truly dependent upon the charge of the particles which are undergoing surface adsorption, then the rate of this process may be a measure of the colloid stability of serum protein. The evidence accumulated from our experiments with

pooled serum show that this may well be the case. The form of the surface tension versus time curves obtained from our experiments on human serum offers convincing evidence that the adsorption process is complex. During the first two hours the surface tension appears to decrease parabolically with a gradually decreasing slope. After the second hour the rate of decrease becomes greater and the surface tension again decreases parabolically and approaches equilibrium at the tenth hour. The complete curve has the appearance of a complex adsorption isotherm, and this indicates that more than one process must be taking place either concurrently or consecutively (Fig. 6).

The findings which appear to be significant at this time are as follows:

a) Refrigerated aliquots of the same serum are reproducible in surface tension values to within 0.1 dyne over a 24 hour period.




The Reproducibility of Surface Tension Measurements on Pooled Serum.

Fig. 6.

b) Measurements show serum from the same apparently normal subjects to be reproducible over periods as long as three months.

c) The serum of fasting young adults exhibits a higher average surface tension at a given time on the surface tension curve than the serum of known atherosclerotics.

d) About thirty per cent of the young adults approach or are within the atherosclerotic range.

e) Increases in the serum surface tension of atherosclerotics have been induced by the intravenous injection of disodium ethylenediaminetetraacetic acid. Such increases persist for weeks following cessation of use of the above compound.

f) The surface tension of pooled serum is increased by heparin and decreased by polyvalent positive ions, or by lowering the pH as measured on the surface tension versus time curve.

If atherosclerosis is promoted by the precipitation of unstable colloidal components, the physico-chemical properties of normal and atherosclerotic serum should be measureably different. From our observations at the present time it appears that this difference is reflected in surface tension values of these different sera.

## *Patient Studies*

### *Method*

5 g of sodium EDTA solution adjusted to pH 7.5 was administered intravenously in 500 $cm^3$ of glucose. The flow was adjusted so that an infusion would require approximately 3 hours. That the drug can be given much faster is brought out in a recent article by *Bechtel, White* and *Estes* (9). It was found that this amount of EDTA was tolerated well 5 times a week for 2 weeks, followed by a 1 or 2 week's rest after which administration was again continued. In most instances patients received between 30 and 40 intravenous infusions or between 150 and 200 g of sodium EDTA.

### *Toxic Reactions*

Gastro-intestinal symptoms are common. Nausea and diarrhea, with occasional severe epigastric pain may occur. Scrotal pruritis has been noted in at least 1 patient. Stomatitis rarely follows in a few days after beginning infusions. 25 to 75 mg of pyridoxine daily eliminates most of these objectionable features. Indeed most patients complain only of a dull pain at the site of infusion which is present only if the administration of sodium EDTA is too rapid. It is routine to test urine daily for any

signs of renal complications. The first 5 days appear to be the difficult period in those patients in whom any of the above symptoms occur. Greater tolerance is experienced after this period of time. The above findings have been gathered from over 5,000 intravenous infusions.

The first patient studied had rheumatic heart disease with a calcified mitral valve. All forms of therapy had failed to alleviate his dyspnea and orthopnea. On a weight basis, the dosage of EDTA administered was much less than that in the rabbit experiments. At the end of 6 days, dyspnea and orthopnea were improved, and after 18 treatments, the patient actually walked up 4 flights of stairs. Following the treatment, the patient's mean pulmonary arterial pressure had dropped approximately one-half. The pulmonary «capillary» pressure was also lowered, but the change was barely sufficient to be considered outside the limits of error inherent in the method. The cardiac output remained constant; therefore, the efficiency of cardiac work improved, since pulmonary resistance fell remarkably.

Shortly after cardiac catheterization the patient shows signs of a pulmonary embolus. His condition grew steadily worse and he met his demise within a few days. Autopsy findings were inconclusive, however, a seriously eroded mitral valve from which particulate matter could be elicited with ease suggested that this was the source of embolic disturbance.

In contrast with this experience a female, aged 43, with severe mitral stenosis was admitted to the hospital through emergency. For 3 years she had experienced increasingly severe dyspnea and for a year had been sleeping elevated in bed or in a chair. At the time of admission she was coughing up blood. She had a choking sensation and pain across the anterior chest with a feeling of severe tightness in the upper chest and throat. Similar spells, but of increasing severity, had occurred during the previous 10 months.

Roentgenograms were taken in June 1955 just prior to sodium EDTA treatment. A calcified valve was visualized on fluoroscopy which also showed up on the roentgenogram. In October the valve could not be detected by either method. In all, the patient received 60 g of sodium EDTA. Presently she is working. Salt restriction and digitalis have been discontinued since October 1955.

One female patient aged 52, with marked xanthelasma was cured of this condition during a course of EDTA.

The following are a few of the case histories of the numerous patients with angina treated by the Providence Hospital group in Detroit.

*Case 1.* W. L., a 50 year old factory worker had angina pectoris in 1949. In February 1954 his attacks had increased so he was using 10 to 15 nitroglycerine tablets a day and walking half a city block produced the pain.

In February he was started on disodium (EDTA) therapy and received 5 g a day for 15 days. A month later there was slight if any symptomatic relief. In March 5 g of disodium (EDTA) was given daily for 15 days. After 7 infusions he was walking about the hospital and using only one nitroglycerine tablet a day. He continued to improve and by mid April was walking half a mile without angina pectoris. A month later he walked a mile without discomfort. From May 1954 to November 1955 he worked regularly and walked a mile or further without symptoms.

*Case 2.* H. V., a 55 year old executive was supposed to have had a coronary occlusion in 1946. He had mild attacks of angina pectoris in June 1952 which occurred with exertion and after eating breakfast. He continued to have occasional attacks of chest pain until September 1953 when they increased so that by July 1954 he was using 8 to 10 nitroglycerine tablets daily. Walking across a room caused angina pectoris.

Beginning in July 1954 he received 29 daily infusions of 5 g of disodium (EDTA) given for 5 days and stopped for 2 days. Since mid September he has been normally active, walked up to 10 blocks without angina pectoris and discontinued the use of nitroglycerine tablets. In October 1955 he had continued with his regular work and had only an occasional slight substernal ache if he exerted unusually following a heavy meal. He had regained his normal strength, vitality and interest in his work.

*Case 3.* J. O., a 59 year old office worker had a myocardinal infarction in 1951. Angina pectoris appeared in February 1953 and progressed so that he had to stop work in September 1953. In January and March 1954 he received a series of 15 daily infusions of 5 g of disodium (EDTA). By the end of February he was walking 6 blocks without angina pectoris. In May he had 8 daily infusions of 5 g of disodium (EDTA), and by July he was free of angina even if doing heavy work. He had remained free of angina when last seen in November 1955.

*Case 4.* E. B., a 54 year old tool maker had angina pectoris in September 1952 which increased so that by November 1953 he could not walk 100 feet without the chest pain. In November 1953 he was given 14 daily infusions of 5 g of disodium (EDTA). One month later he had noticed slight relief.

A series of 14 infusions of disodium (EDTA) was given in January 1954 and in February he was walking several blocks in cold weather without angina pectoris. A series of 15 infusions was given in February and by late March he could walk 3 miles without symptoms.

*Case 5.* E. O'D., a 59 year old night watchman. In 1946 he had severe substernal pain following exertion that lasted 2 hours before relieved by narcotics. In September 1954 he had angina pectoris which increased in frequency and severity until he was forced to stop work in December. He was then given 21 daily infusions of 5 g of disodium (EDTA). By the end of January 1955 he was walking 4 blocks without angina pectoris. A series of 5 daily infusions was given in February after which he returned to work. From March to November he had worked and walked a mile or more without angina.

*Case 6.* L. S., male, age 62, in February 1955 had severe substernal pain following heavy exertion which was relieved by rest. The substernal pain recurred with less exertion or when emotionally upset and was relieved by rest. In May he had to stop work. His blood pressure and heart size were normal but he had auricular fibrillation with a ventricular rate of 80. In May he received 12 daily infusions of disodium (EDTA) given in series of 5 and omitted for 2 days. The therapy was stopped because of diarrhea but he had been relieved of stiffness and pain in his legs and felt stronger. There was no change in the angina pectoris. He received 10 daily intravenous infusions of 5 g disodium

(EDTA) in June, given in series of 5 and omitted for 2 days. After 6 infusions he was walking 8 blocks without angina pectoris. In July his improvement had continued so that he started to work at the factory and was doing work around his home with only a rare and slight substernal sensation which did not require rest. In August he was symptom free. In October the auricular fibrillation was converted to normal sinus rhythm by quinidine sulfate and the ventricular rate was 72. His blood pressure was normal and no heart murmurs were heard. His first electrocardiogram taken in May 1955 had an RS complex in leads V1, V2, V3, and V4 with inversion of the T waves in these leads and also in V5. In the electrocardiogram taken in July the T waves in the V leads were upright, and in that of October the T waves upright in all limb leads. The electrocardiogram in November showed sinus rhythm with normal upright T waves in all leads except AVF.

*Case 7.* F. S., male, age 51, clergyman, in March 1955 had a coronary occlusion. The chest pain recurred with slight exertion but was relieved by rest or nitroglycerine. In September a severe attack had persisted for several hours and radiated into the left arm. The attacks continued to occur with slight exertion, radiating into the neck and left arm until he had to stop work. Walking 200 feet would cause angina pectoris. Beginning in September he received 28 daily infusions of 5 g of disodium (EDTA) in series 5 and omitted for 2 days. By mid October he was free of angina pectoris while walking about the hospital corridors and climbing a flight of stairs. He was able to increase his physical activities, to climb stairs frequently and up to mid December he had remained free of symptoms while performing his normal duties, and walking as far as 10 blocks. His electrocardiograms have remained abnormal throughout the period of observation.

In a group of 20 patients serial electrocardiograms were available for periods of 2 months to 3 years before EDTA therapy. Their electrocardiograms were largely fixed or showed progressive abnormalities. The reversal to essentially normal patterns in a large percent of those without a history of myocardial infarction, but with signs of myocardial damage in the resting state implies a beneficial action due to EDTA.

More specifically of the 20 patients with angina, 14 showed definite and 3 suggestive electrocardiographic evidence of myocardial damage or ischemia. In 6 patients abnormal electrocardiograms reverted to essentially normal patterns during or following EDTA therapy. In 7 patients there was a history of myocardial infarction. While their angina was allayed the electrocardiograms did not change. One subject of this series, a male 68 years of age, died after 15 daily infusions of EDTA.

What is implied in this discussion is that a relationship exists among calcium, magnesium, cholesterol, and mucopolysaccharides. That a relationship exists between calcium and collagen material can scarcely be denied. It would appear that an atheromatous plaque with a collagen-like matrix is not inconceivable. Attack of the calcium of such a matrix may afford one method of successfully minimizing or removing plaques. One is impressed by the report of *Klein* and *Harris* (10), who used sodium EDTA with what appears to be dramatic results in a case of scleroderma with gross calcinosis. From our studies it would appear that serum calcium

level is not the determining factor in the removal of metastatic calcium complexes, but rather the rate of calcium turnover. Presently in our laboratory we are studying this rate with calcium 45 in animals before and after EDTA injections. An increased rate of calcium ion exchange may shift equilibrium in favor of a more normal situation in tissue and against unwanted metastatic deposits.

It seems in order to mention other observations in which calcium and cholesterol appear related. In Hansen's disease calcium in serum is usually high and cholesterol low. Hyperthyroidism is characterized by low serum cholesterol with calcium levels on the high side. The diabetic with acidosis exhibits a high level of serum cholesterol. That calcium as well as sodium and potassium is lost in the urine in this situation is a reasonable assumption. It is known that pregnancy results in a higher serum cholesterol level. During part of the period of gestation a negative calcium balance is prevalent due to fetal demands. Before parturition this imbalance is fully corrected. Several other situations present this inverse relationship.

Rheumatoid arthritis in relation to calcium is of more than passing interest. Rheumatoid lesions have a superabundance of calcium, modified collagen, chondroitin sulfuric acid and other substances. It would seem that calcium is not the least important component of this complex mixture of polymers. Pregnancy in a subject suffering from this condition often brings about a remission of symptoms and, indeed, the process is checked. Are the fetal demands for calcium related to this experience? The demands may play as important a part as the increased elaboration of ACTH from the pituitary which in the non-pregnant patient alleviates symptoms of the condition possibly by stabilizing the collagen picture. It would appear that the pregnant patient has two elements working in her favor: a) increased production of ACTH; b) negative calcium balance.

One last word regarding another situation in which calcium seems to be of paramount importance. Cataracts are common in hypoparathyroidism. Along with this there is often calcification of basal ganglia vessels. In the condition known as pseudo-hypoparathyroidism areas of bone in the skin may develop. All this is accomplished in the presence and in spite of low serum calcium. In other words, low serum calcium due to the removal of parathyroids or fatty infiltration and destruction of the parathyroids (as in the idiopathic type), or the development of resistance to parathormone, as in the case of pseudo-hypoparathyroidism, all result in deposition of metastatic calcium (cataracts, skin, soft connective tissue, brain blood vessels). Actually bones may become more dense.

It is significant that low serum calcium from causes other than parathyroid dysfunction do not produce such lesions. It would not appear unreasonable that most of these lesions (in spite of low serum calcium) are due to the lack of calcium turnover. In other words, the calcium remains too long in the wrong place.

It seems quite likely that normal physiological activity of the parathyroids do more than obviate the unwanted experience of tetany. That there is a relationship between the adrenal cortex and the parathyroids becomes evident in the relatively high incidence of Addison's disease in hypoparathyroidism. Stimulation[1] of the parathyroids would seem to be worthwhile in a study of several of the degenerative diseases.


*Summary*

The use of metal salts of ethylenediaminetetraacetic acid (EDTA) in coronary disease has been found to be of therapeutic value. Absence of anginal pain, increased work tolerance and electrocardiographic evidence appear to indicate that beneficial changes take place in the coronary arteries of treated patients. Serum surface tension studies of treated and untreated subjects display variations which may be related to the colloid stability of serum.

Two cases of mitral stenosis are presented. One, an inoperable mitral stenosis aged 52 years, expired during his hospital stay after having received a course of EDTA and submitting to cardiac catheterization. A second case is one in which rather dramatic fluoroscopic and X-ray changes in the calcified mitral valve were noted. Digitalis has been discontinued in this patient for more than a year.

Animal experimentation using rabbits with induced atherosclerosis indicates that fat and cholesterol metabolism are grossly influenced during the administration of EDTA. Livers, for example, are normal with respect to cholesterol content despite a high cholesterol diet in treated animals as opposed to the livers of control animals. Grossly fewer atheromatous plaques are noted in animals injected with the magnesium salt of EDTA. There is suggestive evidence that the mechanism governing serum calcium levels is stimulated to greater physiological activity in treated animals.

*Zusammenfassung*

Die Verwendung von Metallsalzen der Äthylendiamintetraessigsäure (EDTA) hat sich bei coronaren Erkrankungen als therapeutisch wertvoll erwiesen. Das Fehlen von pektanginösen Schmerzen, steigende Arbeits-


[1] Producing a secondary hyperparathyroidism through a complexation of serum calcium rarely results in the mobilization of calcium beyond the physiological needs (11).

fähigkeit und nachweisbare Besserungen im Elektrokardiogramm scheinen anzuzeigen, daß sich der Befund an den Coronarien der kranken Patienten deutlich bessert. Untersuchungen über die Oberflächenspannung des Blutserums bei behandelten und unbehandelten Patienten zeigen Schwankungen, die vielleicht mit der Kolloidstabilität des Serums erklärt werden können.

2 Fälle von Mitralstenose werden mitgeteilt. Bei dem einen handelt es sich um einen 52jährigen Kranken mit inoperabler Mitralstenose, der nach Behandlung mit EDTA und nach einer Herzkatheterisierung im Krankenhaus verstarb. Bei dem 2. Fall wurden beachtliche fluoroskopische und röntgenologische Veränderungen an der verkalkten Mitralklappe festgestellt. Die Digitalismedikation hatte bei diesem Patienten schon seit über einem Jahr aufgehört.

Tierexperimente an Kaninchen mit experimenteller Atherosklerose zeigten, daß der Fett- und Cholesterinstoffwechsel wesentlich durch EDTA-Gaben beeinflußt werden. Z. B. zeigten die Lebern der behandelten Kaninchen einen normalen Cholesteringehalt trotz einer sehr cholesterinreichen Diät, im Gegensatz zu den unbehandelten Tieren. Im allgemeinen fanden sich bei den mit dem Magnesiumsalz des EDTA behandelten Tieren weniger atheromatöse Veränderungen. Es ist auch deutlich erkennbar, daß bei behandelten Tieren der Mechanismus, der den Serumcalciumspiegel steuert, zu größerer Aktivität angeregt wird.

*Résumé*

On a découvert que les sels métalliques de l'acide éthylène-diaminetétraacétique (EDTA) avaient une action sur les maladies des coronaires. L'absence de douleur angineuse, une amélioration du test à l'effort et l'électrocardiogramme semblent indiquer qu'il se produit une amélioration de l'état des coronaires chez les sujets traités. Des études de la tension superficielle du sérum chez des sujets, traités ou non, montrent des variations, qui peuvent être mises en relation avec la stabilité colloïdale du sérum.

Les auteurs présentent deux cas de sténose mitrale. Le premier, un sujet âgé de 50 ans, atteint d'une sténose mitrale inopérable, est mort après avoir subi un traitement à l'EDTA et avoir été soumis à un cathétérisme cardiaque. Dans le second cas, une image de calcification mitrale impressionnante avait été décelée à la radioscopie et à la radiographie; l'administration de digitale a pu être arrêtée pendant plus d'une année.

Des expériences biologiques sur des lapins, chez lesquels on a réalisé de l'artériosclérose expérimentale indiquent que le métabolisme de la graisse et du cholestérol est fortement influencé par un traitement à

l'EDTA. Par exemple, le foie des animaux traités a une teneur normale en cholestérol, en dépit d'un régime riche en cholestérol, ce qui n'est pas le cas chez les animaux témoins. On voit manifestement moins de plaques athéromateuses chez les animaux à qui l'on a injecté du sel magnésique d'EDTA. Ces faits suggèrent que le mécanisme, qui régit l'équilibre du calcium sérique, est stimulé chez les animaux traités par une plus grande activité physiologique.

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