1976

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THE EFFECT OF DIETARY ADMINISTRATION OF ETHYLENEDIAMINETETRAACETIC ACID UPON THE MINERAL CONTENT OF MOUSE TISSUES

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ABSTRACT

A 3.4 mM ethylenediaminetetraacetic acid (EDTA) aqueous solution and 25 mM Na_2EDTA, Na_4EDTA and $CaNa_2EDTA$ aqueous solutions were fed laboratory white mice as drinking water for 28 day periods. EDTA in the blood plasma of animals used at the end of the 28 day period was undetectable using a colorimetric assay. Ca, Mg and Zn content of bone, kidney, liver and muscle tissues were determined using atomic absorption spectrophotometry. Liver Ca and kidney Mg were reduced in animals administered $CaNa_2EDTA$; kindey Ca and Mg were elevated in animals administered Na_2EDTA; muscle Ca was elevated in animals EDTA; bone, liver and muscle Ca, along with kidney, liver and muscle Zn and bone and liver Mg were reduced in animals administered Na_4EDTA; those animals administered Na_4EDTA also evidenced an elevation of kidney Mg.

INTRODUCTION

Ethylenediaminetetraacetic acid (EDTA) is commonly associated with a general group of chemicals called chelating agents, ligands or coordination compounds. These chelating agents can react with a metallic ion to form a stable complex. The stability of the complex formed is of course a function of the particular metallic ion chelated. The relative standard heats of formation are indicative of the stability constants of the various ligand-ion complexes.

EDTA was first synthesized in 1948 by F. C. Bersworth and was first used therapeutically in the treatment of acute Pb poisoning (Rubin, 1961). The fact that the heat of formation for the PbEDTA complex is very great offers an explanation as to why EDTA is so effective in this treatment. EDTA has since been used in the treatment of Ba, Ca, Co, Cr, Fe, Hg, Mn, Ni and Tl poisoning and also cases of ingestion of radioactive elements such as Th and U (Foreman, 1961).

EDTA is also used commercially as an additive in some foods and beverages. Because metallic ions such as Cu^{++} and Fe^{++} can catalyze reactions leading to deterioration in flavor, coloration, turbidity and vitamin content, EDTA is used as a sequestrant to trap these ions in a nonionic form. Foods which contain oils such as salad dressings are a common source of both $CaNa_2EDTA$ and Na_2EDTA.

Studies involving the mode of action and excretion of EDTA show the

occurrence of pathological changes in certain organs. Intravenous administration of $CaNa_2EDTA$ and Na_2EDTA aqueous solutions in rats and human beings has resulted in a destruction of small intestinal and renal microvilli (Dudley *et al.*, 1955; Foreman, Finnegan and Lushbaugh, 1956; Doolan *et al.*, 1967; Ahrens and Aronson, 1971). Along with structural pathologic changes, an alteration in the permeability of the intestine to normally nonpermeable solutes has been observed with the intravenous administration of aqueous $CaNa_2EDTA$ solutions to rats (Aronson and Rogerson, 1972). Aronson and Rogerson (1972) also indicate that some relationship exists between the presence of EDTA in the body and collagen metabolism.

Less than 20% of an orally administered dose of EDTA is absorbed from the intestine with the largest part being found in the feces (Foreman and Trujillo, 1954; Aronson and Ahrens, 1971). The extent of absorption from the intestine is however a function of the salt of EDTA used. $CaNa_2EDTA$ for example, is absorbed more readily than is CrEDTA (Aronson and Rogerson, 1972).

Foreman and Trujillo (1954) and Foreman, Vier and Magee (1953) have shown that EDTA passed through the body unaltered and was essentially not metabolized in both rats and humans and less than 0.1% of a ^{14}C labelled dose of $CaNa_2EDTA$ was oxidized to CO_2.

Because EDTA is unaltered as it passes through the body, the concentrations of certain metallic ions can be altered (World Health Organization, 1967). Vohra and Bond (1970) found that oral administration of aqueous Na_2EDTA solutions to poultry reduced the Zn content of bone and increased the Zn content of the kidney. The divalent Ca^{++} and Zn^{++} ions are most easily chelated *in vivo* by EDTA (Chenoweth, 1961). Although studies have not dealt with the availability of Mg^{++} *in vivo* chemical data show the stability constant of the MgEDTA complex to be very near the value for the CaEDTA complex.

MATERIALS AND METHODS

White laboratory mice *(Mus musculus* Linnaeus) 40-50 days old were used in all experimental groups. The mice were fed Purina Laboratory Chow *ad lib.* and housed individually in 900 cm^3 galvanized metal cages.

EDTA, $CaNa_2EDTA$, Na_2EDTA and Na_4EDTA were administered orally in the drinking water of the animals. The drinking water of each control group was distilled. Both experimental and control groups were allowed to consume drinking water *ad lib.*.

The concentrations of the aqueous $CaNa_2EDTA$, Na_2EDTA and Na_4EDTA solutions were 25 mM. Because of the low solubility of EDTA in water a saturated solution at 296-K was used (3.4 mM). The determination of the concentrations to be used was made using data from a preliminary acute

toxicity test using $CaNa_2EDTA$. Data from this preliminary test also indicated that the metal content of tissues can be a function of the age of the animal used. For this reason a separate control group was prepared for each experimental group in which the age of animals in each group was the same.

The length of the experimental period for all groups tested was 28 days. Change in body weight and fluid consumed during the experimental period was recorded for each animal. At the end of each experimental period the animals were killed with ether and dissected.

A 0.5-1.0 ml sample of blood was removed from each of 32 mice. Each sample was heparinized and diluted with 10% trichloroacetic acid. The EDTA assay of each sample was then done using the method of Lavender, Pullman and Goldman (1964).

The liver, both kidneys, a portion of the left biceps femoris muscle and the distal part of the left femur were removed and placed in individual metal-free containers. The weight of each tissue sample was determined and each sample was then wet ashed in 5.0 ml concentrated HNO_3. These acidic tissue solutions were then further diluted with water. The Ca, Mg and Zn content of each tissue sample was then determined using a Varian Techtron Model 1000 Atomic Absorption Spectrophotometer. A reducing air-acetylene flame was used in all cases. Slight variations in the length of time which tissues were wet ashed produced comparable changes in the amount of metal liberated. For this reason values for tissue metal concentrations obtained are not necessarily equivalent for groups treated at different times.

Ca, Mg and Zn standard solutions were prepared in equivalent volumes of concentrated HNO_3 and water to minimize unequal ionic interferences.

All chemicals used throughout the study were reagent grade. All water used in EDTA and metal assays was triple-glass-distilled.

RESULTS AND DISCUSSION

The administration of $CaNa_2EDTA$, Na_2EDTA, Na_4EDTA and EDTA in the drinking water of mice used produced no apparent change in the mortality rate compared to that of the control groups.

Differences in the average per cent weight gain did occur. These variations between values for the experimental and control groups are not necessarily indicative of the drinking water treatments employed because the mass of food consumed by each animal was not recorded.

Groups administered $CaNa_2EDTA$ consumed greater volumes of drinking water per g body weight than did the other groups. This may indicate a difference in the palatability of the experimental and control solutions or it may indicate a difference in the volume of water consumed by animals of slightly different ages.

The EDTA assay of Lavender *et al.* (1964) which was used failed to detect any EDTA in the blood plasma of the animals studied. This was true of animals which were administered 25 mM $CaNa_2EDTA$, Na_2EDTA and Na_4EDTA solutions but it was also true of a small group of animals that were administered these solutions at a concentration of 50 mM. This may or may not actually be indicative of the amount of EDTA in the blood plasma of the animals used. A necessary step in the assay involved the precipitation of proteins with 10% trichloroacetic acid. Calcium, magnesium, zinc and iron ions in blood plasma are protein bound to a certain extent (Westerfeld, 1961; Cotzias, 1961; Cantarow and Schepartz, 1967) and if EDTA in blood plasma is associated with any of these metals the removal of protein in the assay would also remove all EDTA associated with the protein.

The Ca, Mg and Zn content of the four tissues studied for each of the experimental and control groups were determined. The significance of variation between the means of each pair of groups was determined using Wilcoxan's signed-ranks test for two groups. Those groups which show significant differences are listed in Table 1. The level of significance for each difference is greater than 95% in all cases.

TABLE 1

TISSUES IN WHICH A SIGNIFICANT DIFFERENCE EXISTS BETWEEN THE MEAN OF METAL CONTENT OF EXPERIMENTAL AND CONTROL GROUPS OF MICE

Drinking water	Tissue	Metal	Type of difference
$CaNa_2EDTA$	Liver	Ca	Reduction
	Kidney	Mg	Reduction
Na_2EDTA	Kidney	Ca	Elevation
	Kidney	Mg	Elevation
Na_4EDTA	Bone	Ca	Reduction
	Liver	Ca	Reduction
	Muscle	Ca	Reduction
	Bone	Mg	Reduction
	Liver	Mg	Reduction
	Kidney	Mg	Elevation
	Kidney	Zn	Reduction
	Liver	Zn	Reduction
	Muscle	Zn	Reduction
EDTA	Muscle	Ca	Elevation

P less than 0.05 in all cases

Kidney tissue is most susceptible to changes in metal content in that changes were found in five cases. Liver tissue appears to be less susceptible to metal alterations than is kidney tissue but more susceptible than both bone and muscle tissues.

Because EDTA is so poorly absorbed from the intestine (Aronson and Ahrens, 1971) observed changes in tissue metal contents are interpreted in terms of the effect of the particular dietary solution which was administered upon the intestinal absorption of metals.

Calcium, in ionic form, is readily absorbed from the upper part of the small intestine and the extent of absorption is a function of many factors. The parathyroid gland mediates the active transport of Ca^{++} in the ileum where vitamin D is a necessary cofactor. This active transport of Ca^{++} which is also Na^{+} and energy dependent functions primarily at low intestinal concentrations of Ca^{++}. Passive absorption is predominant when the Ca^{++} concentration in the intestinal lumen is high. The rate of Ca^{++} absorption is directly proportional to the Ca^{++} concentration and inversely proportional to the Mg^{++} concentration in the intestine. Passive absorption is also related to the pH in the intestine where a lowered pH increases the rate of Ca^{++} absorption (Price, 1961).

Caspary (1972) suggests that a high membrane potential could serve as a driving force for the influx of Ca^{++}. This is especially interesting in light of the work of Hardcastle and Eggenton (1971) which gave evidence of a transient membrane potential increase in everted rat intestines bathed in an aqueous Na_2EDTA solution.

Significant elevations were observed in the Ca content of kidney tissues in those animals administered Na_2EDTA and muscle tissue of those animals administered EDTA. Table 2 shows the pH values of the drinking water solutions used. As mentioned previously, Ca^{++} absorption is pH dependent. Ingestion of some organic acids such as citrate has been shown to markedly increase the rate of Ca^{++} absorption by reducing intestinal pH (Cantarow and Schepartz, 1967). This also seems to be true of the acidic Na_2EDTA and EDTA solutions used in this study. The expected effect of increasing the rate

TABLE 2
pH VALUES OF DRINKING WATER SOLUTIONS

Drinking water solution	pH
0.0034 M EDTA	3.32
0.025 M Na_2EDTA	4.85
0.025 M $CaNa_2EDTA$	6.80
Distilled water	6.90
0.025 M Na_4EDTA	9.80

of Ca^{++} absorption because of an increased membrane potential may also be functioning here but it is not known to what extent this is actually accounting for the increased Ca content of the kidney and muscle tissues mentioned above.

The Ca content of bone, liver and muscle tissues of those animals administered Na_4EDTA and the liver tissue of those animals administered $CaNa_2EDTA$ were all significantly reduced. The ability of each of these two forms to alter the membrane potential in the intestine is unknown. The pH of each of these two solutions is greater than the pH of the acidic EDTA and Na_2EDTA solutions which induced an increase in Ca tissue content. The Na_4EDTA and $CaNa_2EDTA$ solutions which are less acidic seem to have the opposite effect of raising intestinal pH and thus reducing the amount of Ca^{++} absorbed.

Magnesium, like Ca^{++}, is absorbed in the intestine in ionic form but this absorption is not mediated by the parathyroid gland. Absorption of Mg^{++} is linearly related to the lumenal concentration of Mg^{++} and decreases progressively from the proximal to the distal end of the intestine (Aldor and Moore, 1970). Aldor and Moore (1970) also found that 2,4-dinitrophenol fails to inhibit Mg^{++} absorption at high intestinal concentrations of Mg^{++} while the addition of Ca^{++} to the intestinal lumen reduces Mg^{++} absorption.

The Mg content of kidney tissues of animals administered $CaNa_2EDTA$ was significantly reduced. The Ca^{++} and Mg^{++} binding ability of EDTA increases with pH thus Mg^{++} would be bound in greater numbers by the less acidic $CaNa_2EDTA$ solution. Reducing the amount of free Mg^{++} in the intestine would then result in a reduced rate of Mg^{++} absorption.

The Mg content of bone and liver tissues in those animals administered Na_4EDTA were significantly reduced. This observed effect can be interpreted relative to the effect of pH on the intestinal absorption of Mg^{++}. Because Ca^{++} and Mg^{++} are absorbed competitively a common absorptive pathway is suggested. An increase in intestinal pH which reduces Ca^{++} absorption could also reduce the amount of Mg^{++} absorbed.

The kidney Mg elevations found in those animals administered Na_2EDTA and Na_4EDTA may not actually be indicative of increased Mg^{++} absorption. These values indicate that an increased amount of Mg^{++} is being either excreted or absorbed in the kidney which may actually suggest that Mg is being depleted in some other tissue in the body.

Zinc is poorly absorbed from the intestine. Approximately 60% of an administered dose of ^{65}Zn in the intestine was found to be associated with high molecular weight compounds suggesting the occurrence of a carrier-mediated trabsport of some kind (VanCampen and Kowalski, 1971). There is however, evidence to indicate both passive and active transport routes for

Zn^{++} intestinal absorption.

The Zn content of kidney, liver and muscle tissues was altered significantly in those animals administered Na_4EDTA. Reference to Table 1 shows that the Ca tissue content was also altered in many cases for these same animals. Huxley and Leaver (1966) have indicated that a relationship exists between the amount of Ca in the diet of rats and the Zn content of femur and incisors. Rats fed a Ca deficient diet were found to have tissues in which the Zn content was reduced. This relationship between the amount of free Ca^{++} in the intestine and the tissue content of Zn could offer an explanation for this observed effect.

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