Use of Ethylene Diamine Tetra Acetic Acid in Hypercalcemic Patients.* (20645)

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(Introduced by Alfred Gellhorn.)

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Increase in serum calcium concentration not uncommonly accompanies extensive osteolytic metastases. Such hypercalcemia represents inadequate renal excretion of the large amounts of calcium mobilized during rapid bone destruction(1). Compromise of renal function may occur from calcium precipitation in kidney parenchyma or dysfunction without appreciable histologic abnormality; difficulties in calcium excretion may thus be intensified. Efforts to modify hypercalcemia have not been highly effective. Infusion of citrate solutions has not fulfilled the clinical requirement, and decalcification of phlebotomized blood with an ion exchange resin and retransfusion of the hypocalcemic blood has proved cumbersome and inefficacious in our hands(2). Ethylene diamine tetra acetic (EDTA) and its sodium salts (sodium-EDTA) are capable of chelating many metallic ions. The calcium-EDTA complex at pH 7.4 is almost entirely non-ionized and its calcium is non-precipitable by oxalate(3). Accordingly, the calcium atom of the calcium-EDTA complex is physiologically unavailable. Exchange does occur with endogenous calcium(4) and with metals more avidly chelated than calcium(5), but otherwise it is biologically inert. The EDTA molecule itself is metabolically unchanged *in vivo*, and acute toxicity of calcium-EDTA is remarkably low in many different species(6). Spencer and her collaborators(7) have administered 4 g of sodium-EDTA in solution to man over periods of 6 hours, without lowering oxalate precipitable calcium (hereinafter referred to as serum calcium): indeed total calcium per 100 ml was increased by an increment of circulating calcium-EDTA complex. Large amounts of chelated calcium, from 45% to 72% of the stoichiometric equivalent of infused EDTA, were excreted in the urine. In rabbits(8,9) and other laboratory animals(10) when sufficient quantities of parenteral sodium-EDTA are absorbed at a suitably rapid rate, decrease in serum calcium results, leading to asymptomatic hypocalcemia, tetany, or death. Sodium-EDTA has been administered intravenously to 5 patients with far-advanced cancer in whom widespread osteolytic lesions were associated with clinically significant hypercalcemia. Although clinical improvement was not seen, chemical, physiologic and pathologic data associated with the induced hypocalcemic state are of interest, and are herein presented.

Methods. Disodium-EDTA,‡ recrystallized 3 times, was dissolved in distilled water to make a 10% solution. Alkalinization to pH 7.4 was accomplished with 1 N NaOH. After autoclaving and immediately prior to use this was diluted to approximately 3% with 5% dextrose in water. Serum calcium was determined by precipitation with oxalate and oxidation by $KMnO_4$(11) or by hexanitratocerate in excess, employing back titration with ferrous ammonium sulfate to an orthophenanthroline endpoint(12). Calcium bound to EDTA was determined by ashing with concentrated H_2SO_4 at 600°C, followed by oxalate precipitation.

Case 1. 58-year-old female had unoperated breast cancer with extensive osteolytic metastases, symptomatic for 3 weeks. On admission, Mar. 17, 1952, she was anorexic, constipated, lethargic and apathetic. Tendon reflexes were normal, serum calcium 19.0 mg %, phosphorous (P.) 3.8 mg %, alkaline phosphatase (alk. p'tase.) 6.1 Bodansky units (Bod. u.) and NPN 82 mg %. With hydra-

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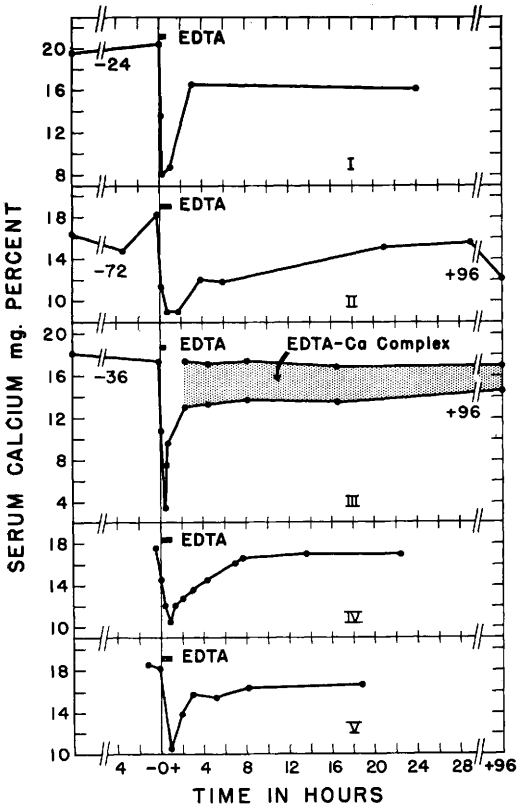

GRAPHS I-V. Response of serum calcium concentration to intravenous administration of sodium ethylene diamine tetra acetic acid.

tion NPN fell to 43 mg % but calcium level unchanged. Urinalysis showed 1+ proteinuria and rare white cells. Administration of EDTA initiated March 25, 25 mg given. Subsequently increasing amounts to 6 g in 45 minutes injected without change in calcium level. Clinical deterioration progressed. On April 10, 400 mg/kg body weight was given. At 15 minutes when approximately 20 g sodium-EDTA had been given (333/mg/kg body weight) she roused from stupor, complained of urge to defecate, of headache, and became restless. Chvostek's sign was negative and there was no hyperreflexia. EDTA was stopped and acute symptoms subsided, with return to obtunded state within one hour. Hypocalcemia occurred, with rapid rebound. (Graph I). Thirty-eight hours after EDTA, while on penicillin, patient expired with hyperpyrexia thought to be due to hypostatic pneumonia. No autopsy was performed.

Case 2. This 49-year-old female had disseminated carcinoma of breast involving soft tissue and causing universal osteolytic and osteoblastic skeletal metastases. On May 7, calcium was 11.4 mg %, P. 4 mg %, and alk. p'tase. 8.3 Bod. u. Admitted May 15, and following day calcium 17.2 mg %, P. 6 mg %, alk. p'tase. 5.8 Bod. u. Urine contained 1+ protein and occasional red and white cells. From May 20 through June 3 she received cortisone .3 g daily by mouth, without appreciable effect on serum calcium levels. NPN 104 mg %. On June 2, because of lethargy, weakness, constipation, and progressive uremia, 15 g of EDTA (approximately 225 mg/kg) were infused in 70 minutes. During infusion no objective changes were noted in sensorium, reflexes or vital signs, and patient did not complain. Serum P. levels remained between 5.2 and 5.6 mg %; changes in serum calcium shown in Graph II. On June 4, dihydrotestosterone 0.1 g daily I.M. begun. Ca. and P. levels returned to normal, alk. p'tase. increased suggesting new bone formation, and uremia subsided. In remaining 3½ months of life neither hypercalcemia nor azotemia recurred. On day of EDTA administration patient's blood count was, as usual, indicative of severe myelophthisic anemia with immature neutrophiles and nucleated red cells in peripheral blood. Platelet count was 120,000 though no previous values were recorded. Without other significant changes in formed elements, on days 4, 7, 9, 14, 18, 25, and 78 after EDTA respectively, thrombocytes (in thousands) were 44, 28, 34, 66, 78, 86, and 250. No changes in bleeding, venous clotting or prothrombin time appeared; epistaxes did occur, however, which were locally controlled. The relation of thrombocytopenia to EDTA, or remission, concomitant with administration of androgen is uncertain. Higher levels of red cells and hemoglobin, without changes in white cells, also occurred coincident with the platelet rise. Demise resulted from pulmonary insufficiency, pathologically attributable to abundant alveolar wall calcification. Metastatic calcification in kidney, although present, was remarkably less extensive. Generalized carcinomatosis was present, though inconspicuous in the lungs.

Case 3. This 49-year-old female had extensive bone destruction from multiple myeloma of 4 years' duration. She remained semi-ambulatory until admission on January 15, 1953, when calcium was 12.2 mg %, P. 3.1 mg %, alk. p'tase. 2.0 Bod. u. and NPN 43 mg %. Her urine findings were: sp. gr. 1.005, acid, protein 4+, and 1-3 white cells/hpf. She remained in bed because of pain, and was treated with 6-mercaptopurine. Increasing serum calcium level was detected during this period which by Feb. 4 was 18.2 mg %. Urinary calcium excretion ranged from 111 to 147 mg per 24 hours. She was lethargic and vomited frequently. 13.5 g of sodium-EDTA (approx. 160 mg/kg) administered intravenously in 29 minutes on Feb. 5. At approximately 25 minutes patient expressed apprehension of death and complained of urge to defecate. Obstructed inspiration and trismus appeared. EDTA was stopped at approximately 29 minutes; respirations ceased and cardiac sounds were not heard. Unsuccessful cardiac puncture seemed to stimulate one respiratory grunt. With a longer needle right ventricular blood was obtained and one cc epinephrine 1:1000 injected. Period of apnea is estimated at 3 minutes, and she regained consciousness in 20 minutes. The ventricular serum calcium was 3.4 mg %. Later venous levels shown in Graph III. In next 16 hours she voided 250 cc, but thereafter daily urinary output was less than 100 cc. Approximately 2.5 mg % of calcium-EDTA was detectable in serum even 96 hours after administration, attributed to the profound oliguria. On fourth day after EDTA, in the 55 cc urine voided, virtually all calcium excreted was complexed with EDTA. In spite of conservative management, patient died on Feb. 11 with acute renal insufficiency, and with progressive leucopenia due to 6-mercaptopurine. No autopsy performed.

Case 4. This 49-year-old female had had symptomatic multiple myeloma for 4 months, with extensive bony involvement, soft tissue tumor of thoracic wall, and proteinuria. One week earlier serum calcium elsewhere was 14.5 mg % and BUN 15 mg %. On admission Apr. 6, the urine showed heavy proteinuria and serum calcium 17.6 mg %, P. 5.2 mg %, alk. p'tase. 2.3 Bod. u., and NPN 67 mg %. In spite of adequate hydration and supportive therapy, on Apr. 15 serum calcium was 20.2 mg % and NPN 171 mg %. On Apr. 16, 10 g sodium-EDTA (approx. 175 mg/kg) given intravenously in 54 minutes. No lessening of torpor nor other subjective reaction occurred. Calcium values are shown in Graph IV. In the remaining 4 days of life there was no alteration in course of her disease. She died Apr. 20 from progressive renal insufficiency. At necropsy, universal multiple myeloma with marked osteolysis was found, and the kidneys showed mild Bence-Jones protein nephrosis. In addition, many tubular cells showed marked vacuolization reminiscent of sucrose nephrosis. Minimal metastatic calcification was present. The marked hydropic degeneration was entirely similar to that found in patient No. 5 (v.i.) and is felt to be independent of other renal pathology present. These changes may have been produced by sodium-EDTA; they are not those recognized as associated with myeloma, amyloid, or hypercalcemia (Fig. 1).

Case 5. 32-year-old female had had malignant melanoma of the rectum for one year despite attempted removal. Inguinal node metastases and extensive destruction of the bony pelvis and lumbar spine had occurred, and she was first admitted for bone pain. Serum calcium was 10.6 mg % on Mar. 30, and on Apr. 21 NPN was 30 mg %. By Apr. 27 calcium was 19.2 mg %, P. 5.4 mg %, alk. p'tase 5.6 Bod. u., and NPN 60 mg %. Urine revealed only 1+ protein. Her condition deteriorated markedly and she became semicomatose. On May 7, 9.5 g of sodium-EDTA (approx. 235 mg/kg) administered intravenously in 60 minutes. Serum calcium dropped as seen in Graph V. In the 4 days until death, hypercalcemia continued and uremia progressed. At autopsy widespread malignant melanoma without ureteral obstruction and intense vacuolization of tubular epithelium were found. (Fig. 2). Metastatic calcification was present in some tubular cells. Intracellular eosinophilic masses, in close juxtaposition to calcified areas were also seen and raise the possibility that cal-

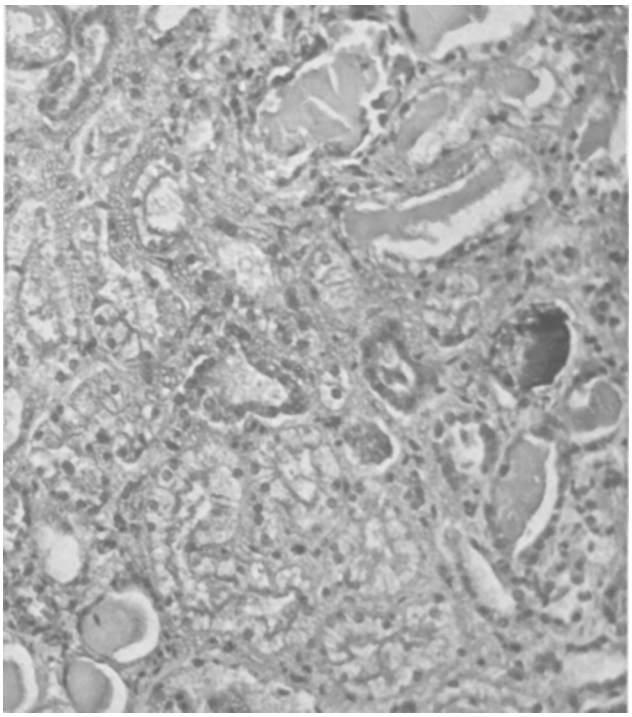

FIG. 1. Hydropic degeneration of renal tubular epithelium in patient #4. Tubules distended with Bence-Jones protein and metastatic calcification also shown.

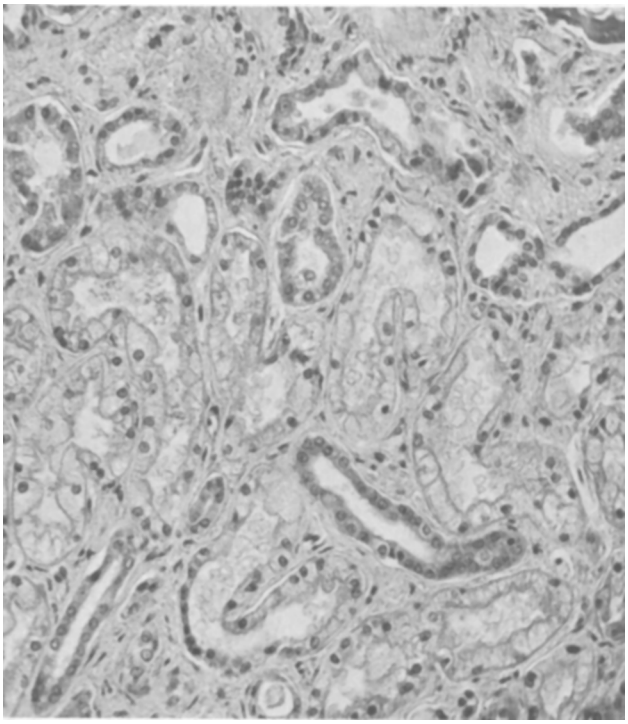

FIG. 2. Vacuolization of tubular epithelium in patient #5. Relatively normal and calcific tubules also shown.

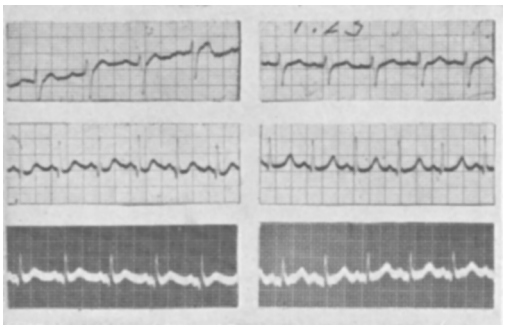

FIG. 3. Electrocardiograms of patients #3, #4 and #5 (top to bottom) during hypercalcemia (left) and normocalcemia (right). Consult Table I. Normocalcemic record in patient #3 taken before apnea. See text.

cium may have been present in these loci before the sodium-EDTA infusion. There was a small amount of metastatic calcification in the lungs, but none in other tissues.

Discussion. The rapidity with which serum calcium levels can be depressed with EDTA at dosage levels employed in this study is noteworthy. As in experimental animals, this is a function not only of amount administered but of rate. There is no reason to assume that homeostasis of serum levels during slow infusion, or elevation toward normal following rapid infusion is a function of dissociation of calcium-EDTA or of renal reabsorption of unbound calcium. Rather, as Spencer and her collaborators(7) indicate, in the presence of excess calcinuria, this homeostasis must represent mobilization of calcium from bone. The speed with which this calcium shift occurs, and its control without apparent overshoot are of interest. The role of the parathyroids in this phenomenon cannot be deduced from data presented, nor is the reason for return to an unphysiologic level elucidated.

Electrocardiograms were taken within one minute of blood specimens during experimental periods in patients No. 3, No. 4, and No. 5. The K in Bazzett's formula $K = \frac{Q\text{-}T}{\sqrt{R\text{-}R}}$ rose slightly in each instance from the control to the depressed calcium level. This is in accord with the generally accepted slowed repolarization during hypocalcemia (Fig. 3, Table I). Initially high K values, the presence of uremia, and the near-moribund state allow little significance to be placed in the small variations observed.

TABLE I. Cardiac Repolarization at Varying Levels of Serum Calcium.

Bazzett's formula $K = \frac{Q\text{-}T}{\sqrt{R\text{-}R}}$.

Patient	Time, min.	Ca, mg %	Q-T, sec.	R-R, sec.	K
#3	0	17.4	.30	.67	.37
	15	10.8	.38	.60	.49
#4	0	17.5	.35	.50	.49
	77	10.5	.38	.56	.51
#5	0	18.2	.32	.53	.44
	67	10.5	.33	.49	.47

The death of patient No. 1 was probably independent of this study. The possibility that thrombocytopenia in patient No. 2 may have been related to the compound cannot be summarily dismissed, although similar reports have not been found. The excessive hypocalcemia achieved in patient No. 3 resulted in a series of events terminating in death. In patients No. 4 and No. 5 the peculiar hydropic degeneration of tubular epithelium found at autopsy, in each instance 4 days after the administration of EDTA, may represent an effect of this agent. In neither instance was this lesion recognizable clinically, nor did it seem to influence the patients' survival. Similar findings have been described after administration of calcium-EDTA to dogs, but untreated and saline-treated control animals were also found to have vacuolization of their renal epithelium(13). The role of EDTA in causation of the lesion in the experimental group was therefore discounted. In rare cases tubular vacuolization has also been observed at autopsy in patients with cancer who have not received EDTA(14,15). The surprisingly little renal calcification in patient No. 2 contrasted to the abundant pulmonary calcification present, admits of the possibility that renal calcification may in some way have been modified by EDTA presented to the kidney for excretion.

Failure to improve the condition of 5 patients with cancer, bone destruction and hypercalcemia by transient depression of serum calcium is not wholly unexpected. In no instance did azotemia regress, nor did the torpor

often ascribed to hypercalcemia remit during the sodium-EDTA infusion except evanescently in patient No. 1. The eventual return of hypercalcemic levels to normal in patient No. 2 is not attributed to EDTA. With the exception of patient No. 3, no apparent modification of survival was effected by the procedure.

Summary. 1. A sodium salt of ethylene diamine tetra acetic acid at pH 7.4 has been administered rapidly intravenously to 5 patients with hypercalcemia from bone destruction. 2. Decrease in serum calcium concentration has been produced but this is of remarkably short duration and unassociated with clinical benefits. 3. Two instances of renal tubular vacuolization following administration of sodium-EDTA have been observed.

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Effect of Electroshock Therapy on Blood Lactic Acid and Pyruvic Acid. (20646)

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Electroshock therapy (EST) is widely used in the treatment of certain mental disorders. However, the biochemical changes in blood constituents resulting from such traumatic electrical stresses to the body are little known. Our immediate interest in this problem was centered around the changes in lactic acid and pyruvic acid levels appearing in the blood after the extreme muscular excitation induced by EST. The evidence available in the literature concerning the effect of EST on the lactic acid and pyruvic acid levels, as well as on other constituents, in the blood is both fragmentary and inconclusive(1-3). In most cases reported, the time intervals of blood sampling of patients receiving EST were chosen arbitrarily and consequently have given rise to conflicting and unsystematic data. We undertook this investigation to ascertain precisely the changes occurring in both lactic acid and pyruvic acid levels of the blood following EST.

Methods. Ambulatory patients, ranging from 20 to 40 years of age and carrying a diagnosis of chronic schizophrenia, were employed in this study. Patients having a relatively quiet awakening after EST were