Calc. Tiss. Res. 16, 1—12 (1974)
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Original Papers

Effects of Ethylenediaminetetraacetate (EDTA) on Urinary Excretion of Hydroxyproline, Calcium and Phosphorus in the Rat

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Received October 10, accepted December 17, 1973

The effects of ethylenediaminetetraacetate (EDTA) were studied in rats. Intravenous infusion of 4.84 mM Na_2EDTA increased the urinary excretion of calcium six-fold, phosphorus three-fold and hydroxyproline 55% in 158 g thyroparathyroidectomized (TPTXed) rats. Calcitonin (25 MRC mU/rat/h) abolished the sodium EDTA-induced increase in hydroxyproline excretion, presumptive evidence that sodium EDTA was acting on bone. To determine whether the changes induced by sodium EDTA are due to lowering of plasma calcium, rats were infused with 4.84 mM ethylenebis(oxyethylenenitrilo)tetraacetic acid (EGTA), a more specific calcium chelator. EGTA increased the urinary excretion of calcium and phosphorus ($P < 0.001$) but not hydroxyproline in thyroparathyroidectomized rats. Furthermore, when TPTXed rats were infused with calcium EDTA (4.84 mM Ca_2EDTA) in order that ionic calcium concentration would not be altered, hydroxyproline excretion was again markedly increased but phosphorus excretion was decreased by 26%. Since the displacement of the sodium ions in Na_2EDTA by calcium *in vivo* is instantaneous, and since calcium EDTA itself induces collagenolysis, the increase in urinary hydroxyproline excretion due to sodium EDTA cannot be attributed solely to lowering of plasma calcium. From these data two conclusions are drawn. First, sodium EDTA enhances bone breakdown independently of its effect on parathyroid hormone secretion. Second, since bone plays a major role in the maintenance of plasma calcium, interpretation of results should be made with caution in those investigations in which EDTA is used to study calcium homeostasis.

Key words: EDTA — Bone resorption — Parathyroid hormone — Calcium — Hydroxyproline.

Introduction

Ethylenediaminetetraacetate (EDTA) has many therapeutic and investigative applications in man and laboratory animals. It has been used to treat lead (Rubin *et al.*, 1953; Andrews, 1961) and iron (Simpson and Blunt, 1960) poisoning, to lower plasma calcium in hypercalcemic patients (Holland *et al.*, 1953; Bessman and Doorenbos, 1957), to test for parathyroid insufficiency (Jones and Fourman, 1963), to produce hypocalcemia in order to study calcium homeostatic control (Copp, 1957; Waron and Rich, 1969) and to evaluate the interrelationship of parathyroid hormone and growth hormone in the regulation of plasma calcium (Gerschberg *et al.*, 1967). Hitherto, in the interpretation of results of investigations of this nature, only the metal-chelating property of EDTA has been considered. Recently, while attempting to lower plasma ionic calcium levels in thyroparathyroidectomized (TPTXed) rats, we observed that intravenous infusion of

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sodium EDTA markedly increased urinary hydroxyproline and phosphorus excretion. Since metal chelates of EDTA cause renal (Foreman *et al.*, 1953; Foreman and Nigrovic, 1968), hepatic (Reuber, 1967) and intestinal (Aronson *et al.*, 1968; Aronson and Rogerson, 1972) lesions, the present study was undertaken to investigate whether EDTA might also affect bone collagen, and if so, determine whether its effect is secondary to lowering of plasma calcium.

Materials and Methods

Male Wistar rats weighing between 129 and 219 g were used. Since EDTA, by lowering plasma calcium might affect the secretion of parathyroid hormone and calcitonin, all animals were surgically thyroparathyroidectomized and maintained on replacement thyroxine (5.0 μg sodium-L-thyroxine/100 g body weight/day, subcutaneously). Only rats with plasma calcium of 3.50 mequiv/l or less two days postoperatively were used in subsequent studies.

Solutions of Na_2EDTA, Ca_2EDTA, ZnEDTA and EGTA were made in 5% dextrose at a concentration of 4.84 mM, and the pH adjusted to between 7.0 and 7.4. Synthetic human calcitonin (Ciba-Geigy Ltd., Basle) with a potency of 100 MRC units/mg was dissolved in neutral 5% dextrose and the concentration adjusted to 8.3 mU/ml. Parathyroid extract (Eli Lilly Parathormone containing 100 USP units/ml) was diluted with 5% dextrose to a concentration of 3.3 USP units/ml and the solution adjusted to pH 4.05 since a precipitate was formed at neutral pH.

Each animal was mounted in a restraining container designed to facilitate collection of urine and a lateral tail vein cannulated. Using a multichannel Technicon autoanalyzer pump, rats were infused at the rate of 3 ml/h. Except as otherwise indicated, animals were infused with 5% dextrose solution for 16 to 20 h before administration of test substances.

Specific Investigations

1. Infusion of Sodium, Calcium and Zinc EDTA. To investigate the effects of metal chelates of EDTA, rats were further infused with 5% dextrose for a control period of 3 or 4 h and then with either sodium, calcium, or zinc EDTA for a similar period.

2. Simultaneous Infusion of Sodium and Calcitonin. These studies were carried out to determine whether the effects produced by EDTA were due specifically to bone resorption since breakdown of skin might contribute to hydroxyprolinuria. Ten rats were infused with sodium EDTA, pH 6.5, for 4 h. Half the animals were then further infused with sodium EDTA while the other half received both sodium EDTA and calcitonin simultaneously for another 4 h. To determine the effects of calcitonin alone, 4 rats were infused with 5% dextrose for 4 h and dextrose and calcitonin for a similar period.

3. Simultaneous Infusion of PTE and Calcitonin. The purpose of this study was to determine whether the effect of calcitonin on parathyroid hormone-induced collagenolysis is similar to its effect on collagen breakdown induced by sodium EDTA. All rats were infused with 5% dextrose for 2 h. Subsequently, half were given parathyroid hormone for 3 h and then parathyroid hormone and calcitonin for further 4 h. The remaining animals were infused with parathyroid hormone for 7 h.

4. Long-term Administration of Calcium EDTA. Two groups of animals were studied. Rats in one group received subcutaneous injections of 21.4 mg of calcium EDTA twice daily. Control animals received solvent vehicle. On the twenty-first day each rat was hydrated by gavage with 10 ml of distilled water, put in a stainless steel metabolic cage, and urine collected for 4 h. Animals were then anaesthetized, bled, and the right femur of each rat dissected out and cleaned of soft tissue. Each bone was broken in two and the medullary cavity washed free of marrow with ice-cooled distilled water. The abdomen of each animal was shaved and a sample of skin free of underlying tissue obtained by dissection. The skin biopsies were fixed, sectioned and stained with either hematoxylin and eosin or Masson. The latter stain identifies collagen.

5. Infusion of EGTA. This investigation was designed to determine whether the effects induced by sodium EDTA were due specifically to lowering of ionic calcium. EGTA is a more

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specific calcium chelator than EDTA (Schmid and Reilley, 1957). Two groups of rats were studied. Animals in one group were infused with 5% dextrose and the others with 5% dextrose containing 5 mmoles Ca/l for 16 h. Subsequently, hourly urine samples were collected for a control period of 4 h. EGTA was then added to the perfusates and the infusion continued for another 4 h.

Collection of Samples

Except where otherwise indicated, urine samples were collected hourly. At the termination of infusion, rats were anaesthetized and blood drawn from the abdominal aorta into ice cooled heparinized tubes. The blood was spun immediately in a refrigerated centrifuge and plasma separated. Plasma and acidified urine samples were frozen at $-20°$ until analyzed.

Analytic Procedures

Plasma and urine calcium were determined by atomic absorption using a Perkin Elmer Atomic Absorption Spectrophotometer Model 403, plasma and urine phosphorus and urine creatinine by the methods described by Rice (1960) and urine and bone hydroxyproline by the method of Kivirikko *et al.* (1967). Urine hydroxyproline, calcium and phosphorus were corrected for creatinine excretion.

Results

Effects of Infusion of Sodium, Calcium and Zinc EDTA. Sodium EDTA increased the urinary excretion of calcium six-fold, phosphorus three-fold and hydroxyproline 55% (Fig. 1a). Compared to controls, plasma calcium was decreased ($P<0.01$) due to formation of calcium EDTA which is readily excreted by the kidneys (Table 1). Although the increase in urinary phosphorus excretion may be due to lowering of plasma calcium (Rasmussen *et al.*, 1967), the effect on hydroxyproline excretion cannot be attributed solely to chelation of plasma calcium by sodium EDTA since in rats infused with calcium EDTA urinary hydroxyproline excretion was also markedly increased (Fig. 1b). However, in contrast to sodium-EDTA infused animals, plasma phosphate and the rate of urinary phosphorus excretion were slightly decreased (Table 1 and Fig. 1b). Infusion of calcium EDTA increased both plasma calcium ($P<0.02$, Table 1) and urinary calcium excretion (Fig. 1b) as would be expected. In rats infused with zinc EDTA urinary excretion of hydroxyproline was unchanged and urinary phosphorus slightly decreased (Fig. 1c), presumptive evidence that the changes induced by sodium and calcium EDTA were not due to the EDTA molecule *per se*. Urinary calcium, however, was slightly increased (Fig. 1c), due presumably to chelation of plasma calcium by monozinc EDTA, although plasma calcium was unchanged (Table 1).

Effects of Infusion of Calcitonin and Parathyroid Hormone. Calcitonin decreased both urinary phosphate and hydroxyproline excretion (Fig. 2). Conversely parathyroid hormone increased phosphorus and hydroxyproline excretion, and induced calcium retention (Fig. 3a). Calcitonin abolished the parathyroid hormone-induced hydroxyprolinuria, but only partially prevented the increase in phosphate excretion (Fig. 3a). These observations are compatible with the known effects of parathyroid hormone and calcitonin and suggest that calcitonin inhibits the osteolytic but not the phosphaturic effect of parathyroid hormone.

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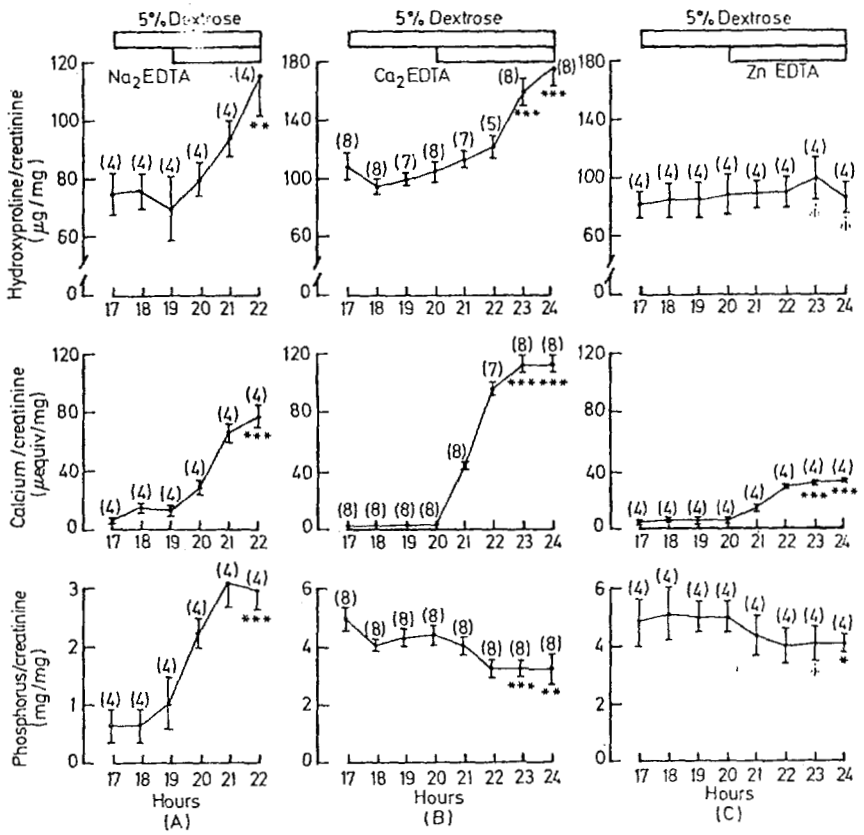

Fig. 1a—c. Effects of EDTA on urinary excretion of hydroxyproline, calcium and phosphorus in thyroparathyroidectomized rats. (a) Sodium EDTA. Animals weighing approximately 158 g were infused with 5% dextrose for 3 h and with 5% dextrose containing 4.84 mM Na_2EDTA for a similar period. (b) Calcium EDTA. Rats weighing approximately 142 g were infused with 5% dextrose for 4 h and with 5% dextrose containing 4.84 mM Ca_2EDTA for a similar period. (c) Zinc EDTA. Rats weighing approximately 145 g were infused with 5% dextrose for 4 h and with 5% dextrose containing 4.84 mM ZnEDTA for a similar period. The rate of infusion in all studies was 3 ml/h and animals were infused with 5% dextrose for 16 h prior to hourly collection of urine samples. Each point indicates a mean value, the vertical bars the S.E.M. and the numbers in parentheses the number of animals studied. Those mean values with symbols were compared by Student's *t*-test with the corresponding mean values of the control period

P = NS; $*P<0.05$; $**P<0.01$; $***P<0.001$

Effects of Simultaneous Infusion of Sodium EDTA and Calcitonin. As in the preceding study, calcitonin abolished the increase in urinary hydroxyproline excretion induced by sodium EDTA and slightly decreased calcium and phosphorus excretion (Fig. 3b). These data indicate that sodium EDTA, like parathyroid hormone, enhances bone breakdown which can be inhibited by calcitonin. However, the effect of calcitonin alone could account for the decrease in urinary

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Table 1. Effects of EDTA on plasma calcium and phosphorus in thyroparathyroidectomized rats

Treatment	Rat wt (g)	P	Plasma calcium (mequiv/l)	P	Plasma phosphorus (mg/100 ml)	P
Dextrose	130 ± 2 (5)	NS	2.30 ± 0.04 (5)	<0.01	9.92 ± 0.56 (5)	NS
Na_2EDTA	129 ± 3 (8)		2.10 ± 0.04 (8)		10.33 ± 0.39 (8)	
Dextrose	138 ± 3 (5)	NS	2.20 ± 0.12 (5)	<0.02	13.20 ± 0.24 (5)	<0.01
Ca_2EDTA	141 ± 2 (7)		2.59 ± 0.08 (7)		12.06 ± 0.20 (7)	
Dextrose	133 ± 2 (4)	NS	2.56 ± 0.23 (4)	NS	13.68 ± 0.99 (4)	NS
ZnEDTA	135 ± 3 (4)		2.64 ± 0.16 (4)		12.20 ± 0.57 (4)	

Rats were infused with 5% dextrose for 4 h and with 4.84 mM Na_2EDTA, Ca_2EDTA or ZnEDTA in 5% dextrose for an additional 4 h. Control animals received 5% dextrose alone for the entire 8 h period

Values are means ± S.E.M. and the numbers in parentheses the number of animals studied

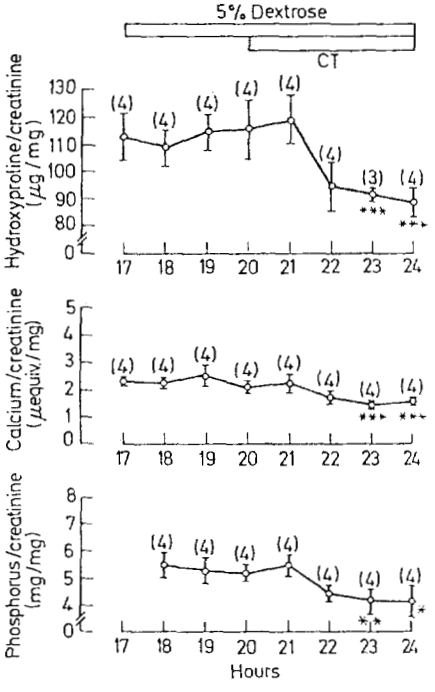

Fig. 2. Effects of calcitonin (*CT*) on the urinary excretion of hydroxyproline, calcium and phosphorus in thyroparathyroidectomized rats. Rats weighing approximately 145 g were infused at the rate of 3 ml/h with 5% dextrose for 4 h and with synthetic human calcitonin (25 MRC mU/rat/h) for a similar period. Animals were infused with 5% dextrose for 16 h prior to hourly collection of urine samples. Each point indicates a mean value, the vertical bars the S.E.M. and the numbers in parentheses the number of animals studied. The mean values marked with asterisks were compared with the means of the appropriate control values by Student's *t*-test

$*P < 0.05$; $**P < 0.01$; $***P < 0.001$

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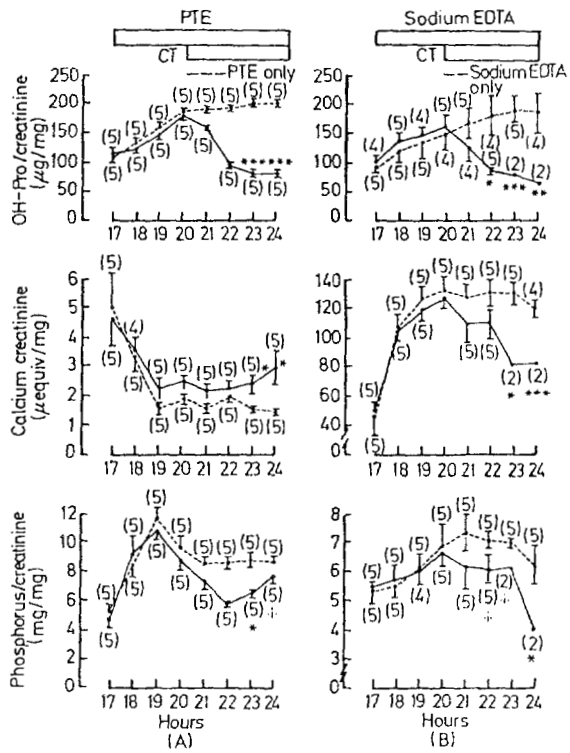

Fig. 3a and b. Effects of calcitonin on urinary excretion of hydroxyproline, calcium, and phosphorus in thyroparathyroidectomized rats infused with parathyroid extract or sodium EDTA. (a) Parathyroid extract (PTE). Rats weighing approximately 147 g were infused at the rate of 3 ml/h with 5% dextrose for 2 h. Subsequently, half were given 10 USP units PTE/rat/h for 3 h and then PTE and 25 MRC mU CT/rat/h for further 4 h. The remaining animals were infused with PTE alone for 7 h. (b) Sodium EDTA. Rats weighing approximately 142 g were infused at the rate of 3 ml/h with 4.84 mM Na_2EDTA in 5% dextrose for 4 h. Half the animals were then further infused with Na_2EDTA while the other half were given Na_2EDTA and 25 MRC mu CT/rat/h for another 4 h. Animals were infused with 5% dextrose for 16 h prior to hourly collection of urine samples. Each point indicates a mean value, the vertical bars the S.E.M. and the numbers in parentheses the number of animals studied. The mean values marked with symbols were compared by Student's *t*-test with the corresponding control values obtained for rats that received PTE or sodium EDTA alone
P = NS; $*P<0.05$; $**P<0.01$; $***P<0.001$

phosphorus excretion in rats given simultaneous infusion of Na_2EDTA and calcitonin (Fig. 4).

Long-term Effects of Calcium EDTA. Administration of calcium EDTA to rats for twenty days induced a greater than two-fold increase in urinary hydroxyproline excretion. Urinary phosphorus excretion was decreased. However, this was not significant when corrected for creatinine excretion (Table 2). Urinary creatinine excretion was 30% lower suggesting that renal function was impaired. Relative to controls, plasma calcium was increased while plasma phosphorus,

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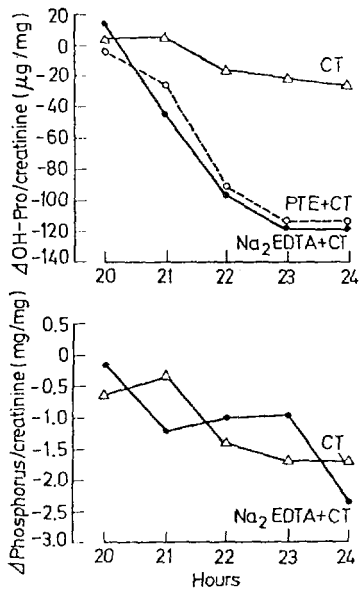

Fig. 4. Effects of calcitonin on thyroparathyroidectomized rats infused with sodium EDTA or parathyroid extract. The values were calculated by subtracting the mean values obtained during calcitonin infusion from the means of the appropriate control values. The triangles denote changes due to calcitonin alone (Fig. 2). The solid and closed circles indicate values obtained in animals given simultaneous infusion of calcitonin and Na_2EDTA (Fig. 3b) or parathyroid extract (Fig. 3a) respectively. Note that calcitonin was more effective in lowering urinary hydroxyproline in parathyroid extract- and Na_2EDTA-infused animals and yet the effect of calcitonin alone could account for the decrease in urinary phosphate excretion in rats given simultaneous infusion of Na_2EDTA and calcitonin

bone calcium and hydroxyproline were decreased. In addition, treated animals grew less quickly. However, the 5.5% difference in body weight between groups at the end of the study cannot account for the 17 and 15% difference in bone calcium and hydroxyproline respectively. The skin of calcium EDTA treated rats contained newly formed fibrous tissue associated with moderate numbers of macrophages and multinucleated foreign body giant cells. This observation suggests that some calcium EDTA was retained at the injection site and induced a foreign body reaction which resulted in a moderate increase in collagen in the granulation tissue. It is therefore possible that the increase in urinary hydroxyproline excretion derived, in part, from skin lesions.

Effects of EGTA Infusion. Urinary calcium excretion was markedly increased in rats infused with EGTA. However, only in rats infused with both EGTA and calcium was this associated with increased phosphaturia. Unlike in rats infused with sodium EDTA, urinary hydroxyproline was not significantly increased (Fig. 5a and 5b). The reason for these difference and their relationship to free plasma calcium concentration is unclear since ionic calcium was not determined.

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Table 2. Effects of long-term administration of calcium EDTA to thyroparathyroidectomized rats

Parameters measured	Controls		Ca_2EDTA treated rats		P
Plasma					
Calcium (mequiv/l)	2.10 ± 0.04	(9)	2.47 ± 0.09	(10)	<0.01
Phosphorus (mg/100 ml)	16.40 ± 0.61	(8)	14.26 ± 0.24	(10)	<0.005
Urine					
Creatinine (mg)	1.119 ± 0.057	(10)	0.783 ± 0.055	(10)	<0.0005
Hydroxyproline (μg)	64.60 ± 4.83	(10)	102.38 ± 9.66	(10)	<0.005
Hydroxyproline (μg/mg creatinine)	57.24 ± 1.61	(10)	130.11 ± 5.77	(10)	<0.0005
Calcium (μequiv)	4.85 ± 0.50	(10)	4.40 ± 0.66	(10)	NS
Calcium (μequiv/mg creatinine)	4.36 ± 0.46	(10)	5.76 ± 0.81	(10)	NS
Phosphorus (mg)	0.344 ± 0.034	(10)	0.243 ± 0.026	(10)	<0.05
Phosphorus (mg/mg creatinine)	0.318 ± 0.043	(10)	0.309 ± 0.024	(10)	NS
Femur					
Calcium (mg)	77.22 ± 1.26	(10)	63.90 ± 2.00	(10)	<0.0005
Hydroxyproline (mg)	7.61 ± 0.09	(10)	6.81 ± 0.14	(10)	<0.0005
Calcium/hydroxyproline (mg/mg)	10.15 ± 0.12	(10)	9.14 ± 0.33	(10)	<0.05

Two groups of animals were studied. Rats in one group received injections of 21.4 mg Ca_2EDTA twice daily. Control animals received solvent vehicle. On the twenty-first day the rats were hydrated by gavage with 10 ml of distilled water, put in stainless steel metabolic cages and urine collected for 4 h. Each animal was anaesthetized, bled, and the right femur dissected out.

Control and Ca_2EDTA treated rats weighed 140 g at the beginning of the study, and 219 and 207 g respectively on the 19th day.

Values are means ± S.E.M. and the numbers in parentheses the number of animals studied.

Discussion

Prior to a discussion of our observations, we would first like to comment on the nature of metal chelators and the sources of hydroxyproline in urine. Ethylenediaminetetraacetic acid combines with metal ions to form stable cyclic structures by covalent rather than ionic bonding. The stability of EDTA complexes increases with the atomic number of the chelated metal ion, and metals are displaced from their complexes with EDTA only by other metals with higher atomic numbers. For this reason the stability of calcium EDTA far exceeds that of sodium EDTA and the affinity of EDTA for lead is many-fold that for calcium (Sidbury, 1955). Ethylenebis(oxyethylenenitrilo)tetraacetic acid (EGTA) is similar to EDTA in its ability to form stable complexes with metal ions. However, it is relatively more specific in its ability to bind calcium (Schmid and Reilley, 1957).

The second comment pertains to the origin of the hydroxyproline in urine. Hydroxyproline is found only in collagen, and bone contains 57% of body collagen (Klein and Curtiss, 1964). When bone is resorbed, both collagen and the inorganic fraction are solubilized (Bates *et al.*, 1962). However, the use of urinary hydroxyproline as an index of bone resorption is complicated by the fact that skin contains 34% of body collagen (Klein and Curtiss, 1964) and like bone, contributes

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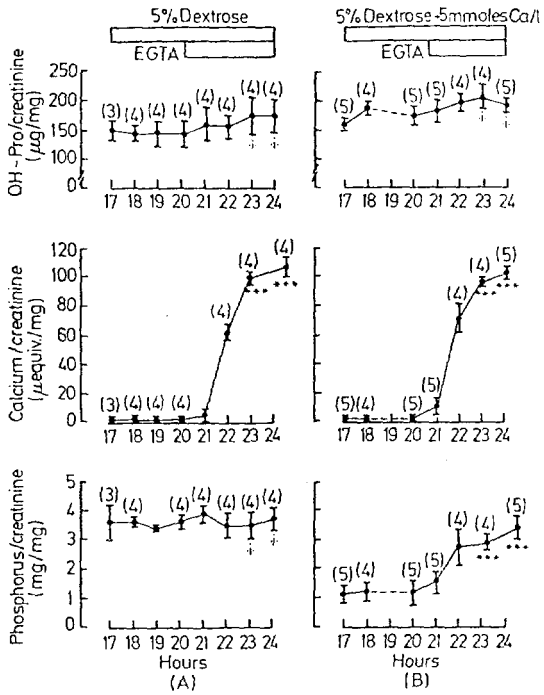

Fig. 5a and b. Effects of EGTA (ethylenebis[oxyethylenenitrilo]tetraacetic acid) on urinary excretion of hydroxyproline, calcium and phosphorus in thyroparathyroidectomized rats. (a) Rats weighing approximately 138 g were infused with 5% dextrose for a control period of 4 h and with dextrose and EGTA for a similar period. (b) Rats weighing approximately 147 g were infused with 5% dextrose containing 5 mmoles of calcium per liter for a control period of 4 h. EGTA was then added to the perfusate and the infusion carried on for a similar period. Infusions were given at a rate of 3 ml/h and animals were infused with 5% dextrose (a) or with 5% dextrose containing 5 mmoles of calcium (b) for 16 h prior to hourly collection of urine samples. Each point indicates a mean value, the vertical bars the S.E.M. and the numbers in parentheses the number of animals studied. The mean values marked with symbols were compared with the corresponding mean values of the control period by Student's *t*-test $P = \text{NS}$; $^{***}P < 0.001$

to urinary hydroxyproline. Furthermore, in addition to breakdown of old collagen, newly formed collagen contributes to urinary hydroxyproline (Laitinen, 1967). It was for these reasons that supplementary investigations were carried out to determine whether the sodium EDTA-induced increase in urinary hydroxyproline excretion was due specifically to alterations in bone resorption.

Our data demonstrate that sodium and calcium chelates of EDTA modify collagen metabolism. Although the mechanism is unknown, at least four possibilities exist. First, sodium EDTA may cause partial decalcification of bone by chelating skeletal calcium, thereby exposing collagen to hydrolytic enzymes. Second, since calcium EDTA enhances permeability of cells (Foreman *et al.*, 1953; Reuber, 1967; Aronson *et al.*, 1968; Aronson and Rogerson, 1972) a similar

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effect on osteolytic cells may alter the concentrations of intracellular ions or enhance the release of hydrolytic enzymes and thereby accelerate osteolysis. Third, since zinc EDTA does not enhance hydroxyproline excretion, chelation of zinc or a trace metal ion lighter than zinc may participate in EDTA-induced collagenolysis. Fourth, sodium or calcium EDTA may interfere with deposition or maturation of newly laid down collagen. In addition, calcium EDTA may act by promoting hypophosphatemia since the infusion of calcium EDTA was associated with lowering of plasma phosphate. Phosphate inhibits bone collagen breakdown *in vitro* (Raisz and Niemann, 1969). Whether decreasing phosphate concentration *in vivo* will have the opposite effects is unknown.

Our findings suggest that sodium EDTA-induced hydroxyprolinuria is due at least in part to increased bone resorption, since it is prevented by calcitonin, a hormone which inhibits bone resorption (Milhaud *et al.*, 1965). That parathyroid hormone-induced hydroxyprolinuria is inhibited by calcitonin in a way similar to that produced by sodium EDTA is further suggestive evidence that both EDTA and parathyroid hormone act upon the same target tissue. However, although parathyroid hormone and calcitonin act on bone, their effects on skin collagen metabolism remain uncertain.

Aronson and Rogerson (1972) reported that calcium EDTA infused into rats whose bones had been prelabelled with [^{3}H]proline thirty days previously increased excretion of tritium labelled hydroxyproline. This finding suggests that calcium EDTA-induced hydroxyprolinuria was due to breakdown of old collagen. However, intact animals and monocalcium EDTA were used in these studies. Therefore hydroxyprolinuria secondary to compensatory hyperparathyroidism stimulated by lowering of plasma ionic calcium induced by formation of dicalcium EDTA cannot be excluded.

Rasmussen and his collaborators (1967) have observed that EGTA infused into thyroparathyroidectomized rats increased excretion of calcium, phosphate and hydroxyproline in the urine. They concluded that EGTA-induced hypocalcemia is the stimulus for both phosphaturia and bone collagen breakdown. Although our findings with sodium EDTA are compatible with this view, there are difficulties in ascribing a hypocalcemic stimulus to sodium EDTA-induced collagenolysis. First, sodium EDTA is almost instantaneously converted to calcium EDTA *in vivo* and calcium EDTA itself induces hydroxyprolinuria. Second, EDTA is not a specific calcium chelator. Furthermore, EGTA, a more specific calcium chelator, markedly increased phosphorus but not hydroxyproline excretion in our studies. Therefore, hypocalcemia *per se* may not be the stimulus for EGTA-induced collagenolysis as has been proposed. The differences in results may be due to the differences in the solutions infused. Rasmussen *et al.* (1967) infused a solution which contained, in addition to the dextrose and calcium used by us, magnesium, sodium, potassium and parathyroid hormone. The addition of EGTA would therefore not only reduce ionic calcium concentration but alter the ratios of the ions present producing changes which might affect cellular or hormonal functions (Pechet *et al.*, 1967). We therefore suggest that provided a judicious choice of infusion constituents is made, EGTA is preferable to EDTA for lowering plasma ionic calcium *in vivo*.

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In conclusion, our data indicate that sodium and calcium EDTA probably enhance bone breakdown. Since bone plays a major role in the maintenance of plasma calcium level, interpretation of results should be made with caution in those investigationsin which EDTA is used to study calcium homeostasis.

Acknowledgements. This work was supported in part by grants from the American Heart Association (71-984) and the U.S.P.H.S. (AM 15462-01). We thank Eli Lilly, Ltd. (Basingstoke), for the bovine parathyroid extract (Para-Thor-Mone) and Ciba-Geigy (Basle) for synthetic human calcitonin used in these studies.

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