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Chelators and Resinous Exchangers. (20120)

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Cation exchange resins of the carboxylic and sulfonic types have found wide application in medical practice for the purpose of sodium removal in cardiac decompensation cases. A major deterrent to a still broader use is the bulk of such agents required for therapeutic efficacy. Clearly, any improvement in capacity would tend to reduce required intake and hence increase patient cooperation.

It was postulated that capacity would be augmented through the removal by chelating agents of polyvalent cations leaving only the monovalent cations for exchange with the resin. Even if this ideal state could be approximated, improvement should ensue. Ethylenediamine tetra-acetic acid or any similar agent will chelate only with polyvalent cations and it is these ions which possess the greater affinity for cation exchange resins, and therefore reduce *in vivo* capacity.

Experimental. The experimental design paralleled in large measure that employed by McChesney and his associates(1-3), Ch'en and Freeman(4). and others. Natrinil, a commercial product, which is a purified cation exchanger carboxylic in a very finely divided state (90% through 200 mesh), 20% in the potassium cycle. and 80% in the hydrogen cycle was used. The *in vitro* capacity was *ca.* 10.0 meq./g. The ethylenediamine tetra-acetic acid was a very pure sample which was used without further purification. The diet had the following composition:

	g
Casein	1500
Salt mix (Hubbel, Mendel, Wakeman)	300
Crisco	1050
Sucrose	3000
Cod liver oil	225
Sodium chloride	75
Yeast	600
Roughage or resin as desired	750
Total	7500

As indicated above, any quantity of roughage may be replaced by resin as the experiment would require. In this way the total quantity of bulk was kept roughly constant. The resin was incorporated into the diet at a 5% level and the EDTA when used at a 1% level.

A pair of rats was placed in each metabolism cage and given the experimental diet and water *ad libitum* for 3 days, were then placed in clean cages and the diet was continued with careful check on the food consumption. The feces were collected every day and pooled until the end of the experiment. The urine was allowed to accumulate in containers containing 3 ml of concentrated nitric acid. The rats were kept on this diet for 4 days and were then placed in clean cages on the same or new diet for the conditioning period (3 days). The collection was made in the usual way. The feces were thoroughly dried at 80-105°C, then ground and thoroughly mixed.

After a review of the literature on flame photometry it was decided to check the results reported by Mosher *et al.*(7) and the agreement was good. Methods were then developed using the idea of radiation buffers as proposed by West *et al.*(8). It was found that the results obtained by this procedure were completely reproducible. Methods for the various elements are summarized briefly below. The urine was checked to make certain that it was

TABLE I. Effect of Ethylenediamine Tetra-Acetic Acid on *In Vivo* Capacity of Cation Exchange Resin. (All values mg/100 g of ingested food.)

	EDTA	Carboxylic cation exchange resin	Combined resin + EDTA	Controls
Fecal Na	86 (4)	127 (5)	346 (2)	75 (20)
	—	123 (10)	176 (10)	51 (19)
	—	175 (7)	245 (7)	67 (20)
	—	164 (7)	211 (7)	—
Urinary Na	536 (4)	388 (5)	476 (2)	458 (20)
	—	312 (10)	202 (10)	350 (19)
	—	249 (7)	189 (7)	309 (20)
	—	244 (7)	194 (7)	—
Fecal K.	110 (4)	138 (5)	409 (2)	114 (20)
	—	124 (10)	234 (10)	84 (19)
	—	139 (7)	273 (7)	81 (20)
	—	127 (7)	221 (7)	—
Urinary K.	593 (4)	674 (5)	823 (2)	595 (20)
	—	571 (10)	405 (10)	436 (19)
	—	478 (7)	398 (7)	438 (20)
	—	503 (7)	460 (7)	—
Fecal Ca	487 (4)	639 (5)	899 (2)	741 (20)
	—	729 (10)	529 (10)	641 (19)
	—	584 (7)	541 (7)	705 (20)
	—	638 (7)	462 (7)	—
Urinary Ca	44 (4)	21 (5)	38 (2)	14 (20)
	—	16 (10)	18 (10)	27 (19)
	—	14 (7)	15 (7)	29 (20)
	—	17 (7)	22 (7)	—
Fecal Cl.	108 (4)	102 (5)	194 (2)	118 (20)
	—	57.8(10)	179 (10)	61 (19)
	—	113 (7)	189 (7)	104 (20)
	—	92 (7)	152 (7)	—
Urinary Cl.	954 (4)	716 (5)	903 (2)	815 (20)
	—	626 (10)	552 (10)	630 (19)
	—	463 (7)	490 (7)	532 (20)
	—	626 (7)	604 (7)	—

Figures in parentheses represent No. of pairs of rats in each exp. leading to avg value stated.

definitely acid and was then diluted to 200 cc (urine stock solution). The "sodium buffer" was prepared by dissolving 0.5 mole each of potassium, calcium and magnesium chlorides in one liter of solution. In a similar fashion the "potassium buffer" was 0.5 molar in sodium, calcium and magnesium chlorides, while the "calcium buffer" was 0.5 molar in sodium, potassium and magnesium chlorides. For the determination of sodium in urine, a standard curve was prepared using 1 ml of sodium buffer per 100 ml and known amounts of sodium chloride. The unknown was then prepared in the same way and its value determined from the standard curve. The method for potassium was similar to sodium except that the potassium buffer was used at the level of 4 ml of buffer per 100 ml. For the analysis of calcium in urine a new method, the validity of which will be established elsewhere, is described. Five ml of urine were pipetted into a 25 cc graduated cylinder; 0.5 ml of calcium buffer was added and then 3.0 ml of 1.0 N ammonium hydroxide. This mixture was diluted to 25 ml, allowed to stand for 10-15 minutes and then filtered. The clear filtrate was read on the flame photometer. The method for chloride in urine was that of Sendroy modified by Van Slyke and Hiller(9). Two g of dry, ground feces were placed in a Vycor crucible, moistened with 1 cc of 6 N sulfuric acid, evaporated to dryness, charred, ashed in a muffle furnace at 500 to 550°C, cooled. One cc of conc. HCl added and the mixture heated. One cc of conc. HNO_3 was added and this was heated. Five ml of distilled water were added and the mixture heated to boiling. It was then diluted to 25

ml and filtered. The filtrate was used for analysis of sodium, potassium and calcium in a manner similar to that described for the urine. The method for chloride in feces was essentially the open Carius method with a modified Volhard-Harvey titration as described by Peters and Van Slyke(10). Phosphorus in the urine and feces was determined as outlined in Hawk, Oser, and Summerson(9).

Results and discussion. From Table I, it is seen that ethylenediamine tetra-acetic acid given alone does not modify sodium, potassium or chloride content but does reduce calcium content of feces. The chelator tends to increase urinary calcium and chloride with no modification in sodium or potassium. In explanation, it is proposed that calcium as a result of its chelation is removed from insoluble salts normally present in the intestine and rendered soluble with resultant absorption and subsequent excretion via the kidney. The facilitation of chloride absorption caused by the EDTA, as reflected in increased urinary excretion, results from reduction by EDTA of calcium cation concentration normally restricting some chloride ions to the intestinal content.

The results reported with the cation exchange resin alone are in large measure similar to those reported by other investigators(1-4) and need no comment. The combination of EDTA with the cation exchange resin compared to resin alone resulted in a marked increase in the capacity of the resin for sodium and potassium removal. Calcium excretion in the feces was decreased by the concomitant administration of EDTA with the resin. The proposed explanation of these findings is that calcium is chelated as when EDTA is given alone and as a result absorbed or rendered non-reactive. This frees the full capacity of the resin for exchange with monovalent cation, and the result is increased capacity. The degree of this effect will be associated with the relative abundance of polyvalent and monovalent cations in the intestinal content, the higher the relative polyvalent ion concentration the more marked will be the potentiation manifested by the EDTA.

EDTA given alone does not modify fecal chloride but does increase urinary chloride. Given with a resin, it modifies the resin effect in that it reduces urinary chloride and increases fecal chloride. It will be recalled that anion exchange resins modify the altered anion excretion caused by cation exchangers in that urinary chloride excretion is decreased and fecal chloride excretion is increased(5,6). Whether or not EDTA reduces the hyperchloremia resulting from cation exchange resin administration remains to be found. It may well be that the combination will reduce the tendency toward the development of acidosis associated with resin usage.

Summary. Ethylenediamine tetra-acetic acid given with a carboxylic cation exchange resin increases the capacity of the resin for monovalent cation removal. A concept is advanced in explanation of this effect.

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