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Effect of Calcium Disodium Ethylenediamine Tetraacetate on Hypercholesterolemic Rabbits (22374)

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The possible mediation of atherosclerosis by the salts of ethylenediamine tetraacetic acid (EDTA) has been explored recently from the standpoint of its effect on cholesterol metabolism(1,2). Curran has demonstrated also that the incorporation of C-14-carboxyl labeled acetate into cholesterol is increased in surviving liver tissue by the addition of 0.0001 M concentration of both the sodium and calcium forms of EDTA(3). It has been reported by Uhl *et al.* that a high cholesterol diet containing calcium EDTA resulted in lower serum cholesterol levels in rabbits than when the compound was administered as the sodium salt subcutaneously(4). In a recent report a contrary view has been advanced(5). Dietary induced hypercholesterolemia in rats was augmented by the inclusion of EDTA compounds in the diet. Further evidence was presented which indicated that parenterally administered EDTA was without effect on endogenous or exogenous cholesterol metabolism in the rat. The present study was carried out to determine whether parenteral calcium disodium EDTA would influence the course of dietary hypercholesterolemia in rabbits.

Materials and methods. Eight rabbits approximately 6 months old and of both sexes of the New Zealand white albino strain were maintained on a high cholesterol diet and water *ad libitum.* The cholesterol diet was prepared by an adaptation of the method of Wang(6) by dissolving one gram of cholesterol (Fisher) in 5.0 ml of ethyl ether (reagent grade, C. P.) and mixing with 100 g of Gleco rabbit pellets. The animals were fed 100 g of diet per day, 6 days per week. After 11 weeks on the high cholesterol diet, the

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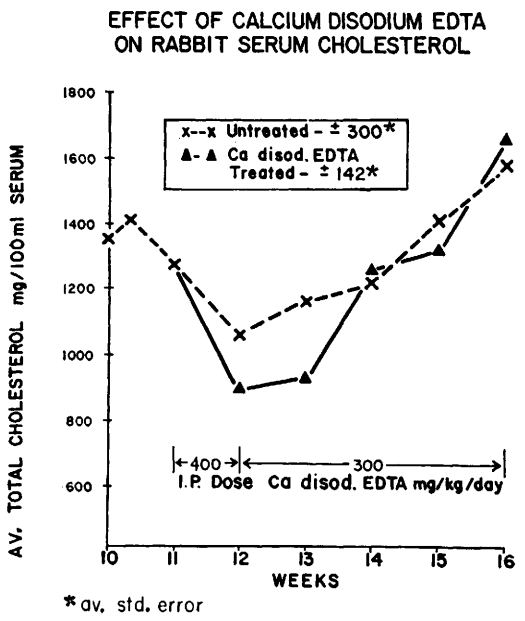

FIG. 1.

animals were divided into experimental and control groups of 4. The experimental group was treated with 400 mg/kg/day of calcium disodium EDTA (Versenes, Inc.) by the intraperitoneal route. After one week the EDTA dosage was decreased to 300 mg/kg/day. Blood for cholesterol analysis was drawn by cardiac puncture. Serum total and free cholesterol levels were determined by the procedure of Zak *et al.*(7). At the cessation of the experiment the animals were sacrificed by air embolism. Aortas and livers were examined for gross evidence of atherosclerosis and fatty infiltration.

Results. The marked hypercholesterolemia of the animals after 11 weeks on the high cholesterol diet is evident by inspection of the data presented in summary in Fig. 1. After one week of treatment with calcium disodium EDTA at a dose level of 400 mg/kg/day the serum cholesterol levels of the treated group decreased sharply. At this dosage level of EDTA the animals were anorexic, their average weight decreased approximately 300 g in comparison to the control group and their food consumption was markedly lowered. When the drug dosage was decreased to 300 mg/kg/day the animals returned to normalcy by the end of one week as indicated by their general behavior and appearance, their increased food intake and their weight recovery toward the level of the control group. The serum cholesterol also returned to the level of the control group after 2 weeks of the reduced EDTA dosage. There were no significant differences in the severity of atherosclerosis and fatty infiltration in the liver as determined by inspection of this limited series of animals.

Discussion. The calcium disodium form of EDTA was selected for this study since in contrast to calcium free sodium EDTA it is without hypocalcemic activity(8). Thus the use of this dosage form of EDTA permitted separation of the metabolic action of EDTA on cholesterol metabolism from its presumed calcium mediating action on the calcium-cholesterol complex. The intraperitoneal route of administration of the chelate avoided the possibility that the known surface active properties of the compound would influence serum cholesterol levels by altering the absorption of dietary cholesterol from the gastrointestinal tract. Further the oral route of chelate administration appeared to rule out the possibility of observation of any direct systemic action of EDTA on cholesterol metabolism since tracer studies have proven the compound to be non-absorbable after oral administration(9).

Results of the present study support the view that EDTA is without effect on cholesterol metabolism. The transient reduction in hypercholesterolemia at toxic levels of the compound may be ascribed to the decrement expected from decreased cholesterol intake in this type of dietary induced hypercholesterolemia. At the maximum tolerated dosage of EDTA there were no significant differences between the serum cholesterol levels of the control and experimental groups.

Summary. Calcium disodium EDTA at the maximum tolerated dosage by the intraperitoneal route had no effect on dietary induced hypercholesterolemia in rabbits.

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Direct Measurement of Arterial Blood Pressure in the Guinea Pig. (22375)

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Search of the literature for blood pressure values in guinea pigs revealed measurements of mean pressure only(1-3) which are unusually low for small warmblooded mammals (4,5) and which give no indication of the magnitude of diastolic and systolic pressures. In view of the increasing use of this species in research and an expressed need for reference values(6) measurements under as normal conditions as possible were made to fill this gap.

Methods. A Statham P-23D pressure transducer and Sanborn amplifier and recorder were used to obtain records of end pressures in the carotid arteries of 8 NIH or Hartley strain guinea pigs which weighed 200-1000 g. The animals were etherized for exposure of the artery and then maintained for an hour under local or general anesthesia as indicated. The cannula was made from a #20 hypodermic needle and polyethylene tubing (i. d. 0.86 mm) and was 14.3 cm long. The pressures recorded during 1-minute periods at 0, 15, 30, 45 and 60 min. were averaged for each animal. Mean arterial blood pressure was calculated as the sum of diastolic + ⅓ pulse pressure.

Results. Table I shows the average and the range of pressures for each animal. Systolic pressures above 100 mm Hg were unusual and occurred briefly and only during activity. The highest recorded was 140 mm Hg in guinea pig #24. The blood pressure was unusually stable during rapid intravenous injection of saline, 6% dextran or 3.5% polyvinylpyrrolidone in dosages of 1% of the body weight. Under general anesthesia the mean pressure tended to be lower than when the animal was conscious and active, and it gradually decreased as time progressed. The blood pressure changed only momentarily in 2 guinea pigs (#8 and #24) when they were placed in the prone position.

Discussion. Since these measurements were made on occluded arteries, the recorded pressures are slightly elevated over the actual values during flow(7). In general, the average rate of descent of late diastolic pressure fell between that of the rat and rabbit in Table II of Woodbury and Hamilton(8). This was also true of the duration of systole and diastole. However, in 14 of the 33 periods in which it was possible to make measurements, the percentage of the cardiac cycle

TABLE I. Carotid Pressures in Guinea Pigs under General or Local Anesthesia. Average and range in mm Hg.

No.	Anesthesia	Systolic	Diastolic	Mean
17	E, P*	71 (28- 88)	39 (16-48)	50 (22-60)
18	Light E	70 (32- 97)	46 (18-90)	55 (23-87)
19	E, Pr	84 (78-120)	56 (33-76)	66 (25-89)
20	Pr	73 (62- 90)	36 (26-58)	49 (40-61)
21	Pr, E	65 (40-108)	37 (22-69)	46 (30-65)
22	Pr	75 (60- 99)	47 (30-72)	56 (43-81)
23	Pr	92 (56-120)	61 (37-77)	72 (44-90)
24	E, Pr	85 (46-120)	54 (30-74)	64 (36-89)
	Avg	76.7	46.8	57.2

* E = Ether; P = Pentobarb.; Pr = Procaine.