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Effect of Dihydrotachysterol (AT10) and Epinephrine on Serum and Tissue Cholesterol and Calcium Levels. (28667)

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Numerous reports(1-5) have appeared implicating calcium deposition with atheroma formation. Since dihydrotachysterol (AT10) has been shown(6,7) to increase serum calcium levels and produce calcification, it appeared of interest to see if an elevated calcium level in the blood together with hypercholesteremia would induce lesion formation.

Intravenous administration of epinephrine, 0.025 mg/kg once daily for 5 days and 0.05 mg/kg twice daily for the next 10 days, has been reported to produce alterations in the aorta of the rabbit(8). Epinephrine administration therefore was included in this study.

Methods and materials. Female Holtzman rats were housed 3 rats per cage, with food and water supplied *ad libitum.* The hypercholesteremic diet (base diet) employed was that previously described(9). It is essentially a high sucrose diet containing 2% cholesterol. To this base diet 400 units per g of Vit. D was added, since it had been found that Vit. D intensified the degree and rate of development of hypercholesteremia in the rabbit(10) and in the rat(9) on a cholesterol diet.

Epinephrine was administered daily 5 days a week by the subcutaneous route to one group of rats at a dose of 100 μg per rat (approximately 563 μg/kg based on the median weight of the rats in the group). In the first experiment AT10, dissolved in corn oil, was injected subcutaneously once a week at a dose of 100 μg per rat (approximately 584 μg/kg) for the first 12 weeks. The dose was increased to 150 μg (approximately 876 μg/kg) for the next 8 weeks. This dose was too high since the rats started to lose weight and 4 deaths resulted.

The dose was reduced to 100 μg for the remainder of the experiment. In the second experiment AT10 was injected as before but at a dose of 125 μg per rat (approximately 600 μg/kg) throughout the experimental period.

The animals were sacrificed by decapitation and a blood sample was taken. A sample of the liver, 100-200 mg from the left lobe, was quickly removed, weighed, and placed into 2N K OH for digestion. The entire aorta was removed, stripped of connective tissue, and weighed. Before digestion it was cut open along its entire length and was examined grossly for lesions. Cholesterol was determined by the method previously described (11). Calcium was determined by a modifi-

TABLE I. Effect of Dihydrotachysterol (AT10) and Epinephrine on Serum and Tissue Cholesterol and Calcium Levels.

Diet and treatment	On diet: No. weeks	On diet: No. rats	Avg wt, g: Initial	Avg wt, g: Final	Food consumed, g/rat/day	Cholesterol concentration: Serum, mg %: Initial	Cholesterol concentration: Serum, mg %: Final	Cholesterol concentration: mg/100 g: Liver	Cholesterol concentration: mg/100 g: Aorta	Aorta wet wt, mg	Calcium concentration: Serum, mg %: Initial	Calcium concentration: Serum, mg %: Final	Calcium concentration: mg/100 g aorta
Normal		12		220			62 ± 3	237 ± 8	177 ± 4			10.0 ±.3	10.2 ± 1.0
Exp 1 (base diet with Vit. D)													
Controls	8	6	146	239	11.0	56 ± 3	170 ± 21	2184 ± 274	160 ± 7	63.5 ± 4.7	10.1 ±.2	11.3 ±.3	7.6 ± 1.8
	28	5	139	201	11.3	49 ± 2	177 ± 24	4605 ± 772	221 ± 7	117.8 ± 8.8	10.9 ±.6	12.8 ±.3	1279 ±435
AT10, 100 μg	8	6	141	211	12.6	52 ± 2	170 ± 21	4521 ± 650*	175 ± 4	64.8 ± 2.0	10.4 ±.5	12.4 ±.6	21.0 ± 5.9*
	28	2	137	196	15.3	59 ± 4	242 ± 29	6869 ±1330	368 ±45*	156.3 ±14.7*	9.4 ±.5	15.2 ±.1*	2937 ± 97*
Epinephrine, 100 μg	8	5	139	216	11.6	46 ± 1	171 ± 15	3216 ± 600	159 ±13	69.7 ± 2.5	10.6 ±.6	12.7 ±.4	10.8 ± 2.8
Exp 2 (base diet without Vit. D)													
Controls	8	6	155	240			227 ± 12	3504 ± 227	173 ± 5	70.8 ± 2.8		9.7 ±.2	13.7 ± .9
	16	6	155	258			190 ± 37	3883 ± 846	168 ± 7	71.2 ± 2.2		8.5 ±.2	11.1 ± 1.1
	24	6	152	284			471 ±115	5485 ± 330	201 ± 7	82.9 ± 3.5		8.1 ±.3	11.1 ± 1.3
AT10, 125 μg	8	6	148	243			214 ± 38	3816 ± 375	166 ± 4	72.0 ± 2.4		11.1 ±.5*	14.8 ± .3
	16	6	154	288			262 ± 29	3724 ± 377	164 ± 5	74.9 ± 1.8		9.1 ±.4	19.1 ± 1.1*
	24	6	154	274			400 ± 68	5753 ± 618	†220 ±18	85.5 ±58.0		9.3 ±.4*	†32.9 ±10.0*
									309				538

* Significantly different from controls at P = .01.

† One rat out of this group had a very high aorta cholesterol and calcium concentration as compared to the other 5 and is therefore given separately.

cation(12) of the method described by Ashby(13).

Results and discussion. It is evident from the data presented in Table I that the base diet, with or without Vit. D, elevated serum and liver cholesterol levels after 8 weeks of feeding. A further increase occurred with continued feeding. Serum cholesterol levels were higher in the second experiment than in the first. This was not expected in view of the earlier findings(9) which indicated that the addition of Vit. D aggravated cholesterol levels. The reason for this is not apparent. No significant differences were found in liver cholesterol levels between the 2 experiments, indicating that Vit. D had little effect on cholesterol deposition in this tissue. Vit. D slightly but significantly increased serum calcium levels, an effect which was aggravated by AT10 dosing. AT10 had no effect on serum calcium in the rats not receiving Vit. D.

Subcutaneous injection of 100 μg of epinephrine 5 days a week was without effect on cholesterol levels and aorta calcium. Serum calcium was slightly elevated. Examination of the aortas showed no gross pathology. It is possible that more intensive dosing with epinephrine would have resulted in aorta pathology, but unfortunately this was not done.

Vitamin D produced aortic calcification after 28 weeks of feeding and this was increased by AT10 administration as indicated in Experiment 1. The same diet without Vit. D, Experiment 2, produced no increase in the calcium level of this tissue, but again AT10 administration resulted in aortic calcium deposition. It is therefore evident that both AT10 and Vit. D can produce aortic calcification independently and augment calcium deposition when given together.

The results indicate that there is an association between calcium and cholesterol deposition in the aorta. In Exp. 1 after 8 weeks there was no significant increase in aorta cholesterol of either the control rats or those receiving AT10. Aorta calcium was slightly increased in the latter group. Intense calcification was observed after 28 weeks, the group receiving AT10 showing the highest calcium level. Aorta cholesterol was moderately increased, the greater increase coinciding with the higher calcium level. The results appear to indicate that calcification may be a predisposing factor for cholesterol deposition. In Exp. 2 feeding the base diet without Vit. D for 24 weeks resulted in an increase in aorta cholesterol without calcification. A high serum cholesterol level accompanied the increase in aorta cholesterol. AT10 administration produced calcification with a greater increase in cholesterol deposition. Examination of the aortas showed no gross atherosclerotic lesions whereas calcification of some of the aortas was obvious.

Summary. A hypercholesteremic diet was fed to rats for up to 28 weeks. Additional dosing of Vit. D, epinephrine or dihydrotachysterol (AT10) had no pronounced effect on serum or liver cholesterol levels. Both Vit. D and AT10 produced a slight increase in serum calcium and a pronounced increase in aorta calcium deposition. There was a moderate increase in aorta cholesterol which correlated with aorta calcification.

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