STUDIES ON THE PREVENTION OF CHOLESTEROL ATHEROSCLEROSIS IN RABBITS

I. The Effects of Whole Thyroid and of Potassium Iodide

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For 25 years the atherosclerosis produced in herbivores by feeding cholesterol or cholesterol-rich foods has attracted the attention of investigators. The literature, already of formidable proportions, has been recently reviewed by Anitschkov (1). The work to date has been concerned chiefly with the production and study of the lesions. Our interest has been in their prevention.

The rabbit as an experimental animal for the study of aortic disease is viewed with suspicion by some workers, largely because of their failure to distinguish clearly between intimal and medial involvement of the aorta.

The lesions produced by feeding cholesterol are intimal, appearing as elevated, yellowish plaques most commonly in the arch, ascending aorta, and around the mouths of the intercostal arteries. When extensive, the process often involves the aortic valve. Calcification of the plaques may occur. The accumulation of large, fat-laden cells beneath the endothelium is a prominent histologic feature. By some pathologists these intimal lesions are considered the analog of human atherosclerosis.

On the other hand, medial lesions of the rabbit's aorta have been produced by a variety of agents such as adrenalin, barium chloride, thyroxin, viosterol, bacterial products, nicotin, and many other substances. The process is characterized by a degeneration of the muscle

cells in the media often followed by a deposition of calcium. The intima is involved secondarily if at all.

The importance of distinguishing sharply between these two types of involvement is due to the high frequency of spontaneous medial degeneration, while spontaneous intimal lesions are rare. In fact it is doubtful whether the latter ever occur in young rabbits.

Control Observations

Before studying the prevention of atherosclerosis it was necessary to be certain that an effective method for the production of these intimal lesions was being employed.

The rabbits used throughout this work were mainly of the Dutch belted variety, although a few rabbits of other breeds were necessary to fill in groups from time to time. The animals were from 4 to 6 months old at the beginning of each experiment. They were kept in individual cages indoors under similar conditions, and were fed a diet consisting of oats, an alfalfa-molasses mixture, and fresh vegetables. As the work has been in progress practically continuously since Oct., 1930, it was possible to rule out a seasonal effect and none, in fact, was noted.

The method of administration of cholesterol favored by most investigators has been to give the substance dissolved in a warm vegetable oil by stomach tube. This method was tried at first but soon abandoned because several rabbits developed inhalation pneumonias, while others stopped eating normally after the oil. It was finally decided to mix 1 gm. of crystalline cholesterol with each rabbit's grain three times a week. The disadvantages of this method are its wastefulness and a lack of knowledge as to the exact cholesterol intake. The advantages, however, are its simplicity, freedom from trauma, lack of interference with normal eating habits, and—not the least consideration—its effectiveness.

In the first test of this method, a group of 15 young rabbits was used. Seven of these were kept as controls and received only a regular diet. Eight rabbits were given cholesterol, at first by stomach tube, later simply mixed with the grain.

In a second group were 16 rabbits, also 4 to 6 months old. Eight of these were used as controls, and 8 were given cholesterol as above. In addition each of the 16 animals had 5 drops of viosterol 250D (Squibb) instilled into the pharynx three times a week. It was thought that viosterol in this dosage might hasten or increase the severity of the changes produced by cholesterol. In so far as aortic lesions, whether intimal or medial, were concerned, the viosterol appeared to be entirely inert. Accordingly, for convenience of consideration, it seemed justifiable to combine the first and second groups. The results were as follows:

	No. of rabbits	Duration of experiment	Cholesterol intake*	Atherosclerosis	
				Present	Absent
		days	*gm.*		
Controls.....	15	92–113	0	0	15
Cholesterol-fed....	5	9– 53	2–10	0	5
	11	87–114	25–37	11	0

* It must be emphasized that figures given for cholesterol dosage represent the maximal possible intake. Due to the limitations of the feeding method used, it is not only possible but probable that the actual intake was less than the amount given in each instance.

No atherosclerosis was found in the aortas of the 15 control rabbits either grossly or microscopically.

It has long been recognized that a time factor is important in the production of the lesions that occur after cholesterol feeding. In the cholesterol-fed group 5 rabbits died in 9 to 53 days, having received approximately 2 to 10 gm. of cholesterol. There was no atherosclerotic change in the aortas. The remaining 11 animals were killed after 87 to 114 days and a cholesterol intake of 25 to 37 gm. Slight to marked macroscopic atherosclerotic change was present in the aorta of each of the 11 rabbits. Involvement of other arteries was also noted in most of the animals together with characteristic changes in such organs as the liver, spleen, kidneys, and adrenals, but, as these lesions have been adequately described by previous investigators and as they do not fall within the restricted scope of the present report, detailed references to them will be omitted.

As the result of this preliminary work it was evident that gross atherosclerosis could be produced in the aortas of 4 to 6 month old rabbits in approximately 90 days by simply mixing crystalline cholesterol with the grain. It is probable that the lesions may appear in a shorter time, but a minimal survival period of 90 days was arbitrarily established as a requirement for inclusion in the series that follow.

Effect of Whole Thyroid and of Thyroxin

In view of the clinical observation that the blood cholesterol tends to be low in hyperthyroidism with a corresponding tendency to abnormally high levels in myxedema, it was decided to try the effect of thyroid given concurrently with cholesterol upon the production of atherosclerosis. It was further decided to test the efficacy both of a whole gland product and of thyroxin.

Control Group: Cholesterol-Fed.—This group contained 21 rabbits from 4 to 6 months old. Cholesterol was added to the diet as in the preliminary work.

Seven animals died in 45 to 83 days and were discarded. Three of these had atherosclerotic lesions at 57, 69, and 83 days respectively.

Fourteen rabbits were killed after 90 to 118 days. The results are given in Table I. Gross atherosclerotic lesions of the aorta were present in every case. A determination of the whole blood cholesterol by the method of Bloor, Pelkan, and Allen (2) was made in 11 rabbits from blood obtained shortly before the animal was killed. Cholesterol values in the blood ranged from 326 to 790 mg. per 100 cc., and averaged 520 mg. By the same method the blood cholesterol of 25 normal rabbits of a comparable age was found to average 105 mg. with a range of 82 to 169 mg.

TABLE I

Cholesterol-Fed Rabbits Used as Controls for Thyroid Series

Rabbit No.	Sex	Weight		Duration of experiment	Cholesterol intake	Blood cholesterol*	Atherosclerosis of aorta	Remarks
		Start	End					
		kg.	*kg.*	*days*	*gm.*	*mg. per 100 cc.*		
A1-4	F.	1.3	1.8	90	35	381	Moderate	
A1-0	M.	1.7	1.8	98	38	532	Marked	
A1-1	M.	1.8	1.8	98	38	326	Slight	
4-6	M.	2.2	2.0	108	39	—	Marked	
4-8	F.	1.8	1.8	108	39	—	Marked	
A7	F.	1.5	1.7	113	44	397	Slight	
A8	F.	2.1	2.4	113	44	543	Marked	
A1	M.	1.7	1.8	114	44	423	Moderate	O_2 consumption
A2	M.	1.4	1.7	114	44	545	Marked	per gm. per hr.
A3	F.	1.5	1.8	114	44	588	Moderate	0.472 cc. (aver
A4	F.	1.9	2.1	114	44	790	Marked	age)
A5	M.	1.6	1.9	114	44	484	Slight	
A6	F.	1.8	2.1	114	44	714	Marked	
5-0	M.	1.6	2.1	118	39	—	Marked	
Average.....						520		

* In a series of 25 normal rabbits the average blood cholesterol was found to be 105 mg. with a range from 82 to 169 mg.

The Effect of Whole Thyroid.—Thirty rabbits were used in this group. Each animal received approximately 0.4 gm. of dried whole thyroid (Parke, Davis) as a powder together with 1 gm. of cholesterol mixed with the grain three times a week. Again it must be pointed out that, as in the case of the cholesterol, the dosage represents the maximal possible intake. The actual amount ingested was almost

certainly somewhat less. The rabbit exhibits a surprising tolerance to thyroid given by mouth. Weight loss, however, was usually marked in this series. One lot of animals was practically wiped out by a protracted period of hot weather that occurred during the thyroid feeding, and it was necessary to intermit the feeding of the survivors (Nos. 7-3, 7-4, 7-5).

Eleven rabbits in the group died in 30 to 77 days, having received 11 to 28 gm. of cholesterol and 5.2 to 10.8 gm. of thyroid. No gross atherosclerosis was present. These animals were excluded from the series.

TABLE II

Effect of Whole Thyroid upon Production of Atherosclerosis by Cholesterol

Rabbit No.	Sex	Weight		Duration of experiment	Cholesterol intake	Thyroid intake	Blood cholesterol	Atherosclerosis of aorta	Remarks
		Start	End						
		kg.	*kg.*	*days*	*gm.*	*gm.*	*mg. per 100 cc.*		
7-3	F.	1.9	1.3	90	28	10.8	—	0	
7-4	F.	1.7	1.4	90	28	10.8	—	0	
7-5	F.	1.9	1.3	90	28	10.8	—	0	
A2-4	F.	1.7	1.3	90	35	14.0	—	0	Died; hyperthyroid?
A1-8	M.	1.9	1.1	93	36	14.4	—	0	Died; hyperthyroid?
A2-1	M.	1.7	1.2	95	37	14.8	205	0	
A2-8	M.	1.6	1.1	95	37	14.8	140	0	
A3-1	F.	1.9	1.1	95	37	14.8	114	0	
A2-7	F.	1.7	1.4	98	38	15.2	291	Slight	
A1-5	F.	1.9	1.3	100	39	15.6	261	Moderate	O_2 consumption per gm. per hr. 0.799 cc. (average)
A1-6	M.	1.6	1.4	100	39	15.6	103	0	
A2-2	M.	1.8	1.1	100	39	15.6	205	0	
A1-7	F.	1.1	0.9	107	42	16.8	104	0	
A1-9	M.	1.9	1.5	107	42	16.8	147	0	
A2-5	F.	1.6	1.2	107	42	16.8	154	0	
A3-0	F.	1.7	1.2	107	42	16.8	132	0	
A2-0	M.	1.9	1.2	108	43	17.2	202	0	
A2-3	M.	1.4	1.4	108	43	17.2	167	0	
A2-6	F.	1.7	1.2	108	43	17.2	263	0	
Average							178		

Nineteen animals were killed or died in 90 to 108 days. The results are shown in Table II. The cholesterol intake varied between 28 and 43 gm., and the thyroid from 10.8 to 17.2 gm. Atherosclerosis of the aorta was absent in 17 rabbits, while gross lesions were present in 2. In 1 of these (No. A2-7) the process was limited to a few small plaques in the descending thoracic aorta. The other rabbit (No. A1-5) showed widespread thickening of the aortic intima, but the plaques were usually discrete and did not coalesce.

The whole blood cholesterol, determined in 14 animals shortly before they were killed, ranged from 103 to 291 mg. per 100 cc. with an average of 178 mg. Both rabbits with atherosclerotic plaques in the aorta had a hypercholesterolemia. One animal also had a considerably elevated blood cholesterol (263 mg.) but no aortic lesions.

The Effect of Thyroxin.—Each rabbit in the preceding group received a maximum of 1.2 gm. of whole thyroid per week. This contained 1.4 mg. of thyroxin. It was desirable to give each animal in the present group an equivalent amount of thyroxin. However, it was decided to give 1.2 mg. instead of 1.4 mg. because the whole amount was to be injected subcutaneously, and because the thyroid-fed rabbits probably

TABLE III

Effect of Thyroxin upon Production of Atherosclerosis by Cholesterol

Rabbit No.	Sex	Weight		Duration of experiment	Cholesterol intake	Thyroxin intake	Blood cholesterol	Atherosclerosis of aorta	Remarks
		Start	End						
		kg.	*kg.*	*days*	*gm.*	*mg.*	*mg. per 100 cc.*		
A3-3	M.	1.4	1.0	90	35	16.8	317	Slight	
6-3	M.	2.7	2.0	94	29	12.0	—	Marked	
A3-9	M.	1.8	1.3	98	38	16.8	368	0	
A3-2	F.	1.7	1.7	107	42	19.2	381	Marked	
A3-6	M.	1.6	1.7	107	42	19.2	170	0	
A3-8	M.	1.7	1.4	107	42	19.2	348	Moderate	
6-1	M.	2.4	1.7	108	39	13.2	—	Moderate	
6-4	F.	2.1	1.7	108	39	13.2	—	0	
A4-0	M.	2.1	1.6	112	44	19.2	658	Moderate	
A4-1	M.	1.6	1.3	112	44	19.2	379	Marked	
A4-2	M.	1.8	1.4	112	44	19.2	568	Moderate	O_2 consumption per gm. per hr. 0.626 cc. (average)
Average							399		

did not eat all of the dried gland that was mixed with their food. Therefore the slightly smaller dose seemed more nearly equivalent.

Accordingly, each rabbit in this series was given the usual gram of cholesterol three times a week, and once a week received a subcutaneous injection of 1.2 mg. of thyroxin (Squibb) dissolved in N/50 NaOH.

Of the 17 animals comprising this group, 6 died in 44 to 80 days and were excluded from the series. The aortas of 5 of these rabbits were normal. The sixth rabbit, dying after 44 days and having received 17 gm. of cholesterol and 7.2 mg. of thyroxin, showed early, diffuse intimal lesions.

Eleven rabbits were killed in 90 to 112 days. The results are shown in Table III. Eight had slight to marked atherosclerotic changes in the aorta. The remaining 3 animals had normal aortas both grossly and microscopically.

The blood cholesterol in 8 rabbits varied from 170 to 658 mg., and averaged 399 mg. All the animals with aortic lesions had a hypercholesterolemia. Rabbit A3-9 had a blood cholesterol of 368 mg. but a normal aorta. This, in our experience, is an unusual combination. Another rabbit (No. A3-6) without atherosclerosis had a relatively normal blood cholesterol of 170 mg.

Discussion.—The facts presented in Tables I, II, and III may be summarized as follows:

Group	No. of rabbits	Atherosclerosis		Average blood cholesterol
		Present	Absent	
				mg.
Cholesterol	14	14	0	520
Cholesterol + thyroid	19	2	17	178
Cholesterol + thyroxin	11	8	3	399

The effectiveness of whole thyroid when given simultaneously with cholesterol in preventing the atherosclerosis and hypercholesterolemia caused by the latter is striking. The comparative ineffectiveness of thyroxin is equally marked. There is no obvious explanation for this difference in response, although the opportunity it affords for speculation is abundant.

A similar effect of thyroid was noted in 1918 by Murata and Kataoka who, in the transactions of the Japanese Pathological Society, reported that they had observed that the experimental arteriosclerosis produced by lanolin feeding was absent or slight in degree when thyroid feeding was combined with the lanolin (3). No amplification of this brief statement has been found.

Another point of interest has to do with oxygen consumption. This was determined on 1 animal in each of the 3 groups through the kindness of Dr. Dickinson W. Richards, Jr. In spite of a substantial elevation of the basal oxygen consumption of both Rabbits A1-5 (Table II) and A4-2 (Table III), *i.e.* a good thyroid effect, gross atherosclerosis of the aorta was present, and the blood cholesterol was 261 and 568 mg. respectively.

Effect of Potassium Iodide

Potassium iodide has long been used empirically in patients with arteriosclerosis and has occasionally seemed of real benefit. Therefore

it was decided to try the effect in a group of rabbits of giving potassium iodide simultaneously with cholesterol.

Because the efficacy of the cholesterol feeding method in causing the appearance of the aortic lesions had already been demonstrated it seemed permissible to use a smaller control group than had been employed previously. Seven rabbits were set aside as controls and were given a gram of cholesterol three times a week according to the usual method. Unfortunately only 3 of these animals survived the requisite period of 90 days. These 3 rabbits were killed on the 98th day, having received 39 gm. of cholesterol. The blood cholesterol was 658, 694, and 472 mg. respec-

TABLE IV

Effect of KI upon Production of Atherosclerosis by Cholesterol

Rabbit No.	Sex	Weight		Duration of experiment	Cholesterol intake	KI intake	Blood cholesterol	Atherosclerosis of aorta
		Start	End					
		kg.	*kg.*	*days*	*gm.*	*gm.*	*mg. per 100 cc.*	
A7-9	F.	1.8	2.1	99	39	39	197	0
A8-0	M.	2.1	2.1	99	39	39	154	0
A8-1	M.	1.5	1.8	99	39	39	138	0
A8-2	F.	1.9	1.8	99	39	39	185	0
A8-3	F.	2.0	2.1	99	39	39	135	0
A8-4	F.	1.4	1.6	100	39	39	240	0
A8-5	F.	2.2	1.9	100	39	39	248	Marked
A8-6	M.	2.1	1.9	100	39	39	136	0
A8-7	M.	1.5	2.0	100	39	39	255	0
A4-4	F.	1.6	1.8	112	44	44	169	0
A4-5	M.	1.5	1.6	112	44	44	111	0
A4-6	M.	2.3	2.3	113	44	44	231	0
Average							183	

tively. Atherosclerosis was evident in the aorta of each animal. In 1 it was classed as moderate in degree, while in 2 it was marked. Atherosclerotic changes were also present in 2 rabbits dying in 49 and 78 days respectively. In 2 other animals dying at 39 and 40 days no lesions were present.

The Effect of KI.—Twelve rabbits were fed a gram of cholesterol and a gram of potassium iodide—the latter in aqueous solution—three times a week. The animals in this group were killed after 99 to 113 days (Table IV). Atherosclerosis of the aorta was absent in 11, and present in only 1. It was, however, marked in this instance. The blood cholesterol varied between 111 to 255 mg. with an average of 183 mg. In 4 animals over 200 mg. of cholesterol per 100 cc. of blood were present, and in 3 of these no atherosclerosis was found. The only rabbit in the series to develop atheromata had a blood cholesterol of 248 mg.

The Effect of KBr and KCl.—To determine, if possible, whether the inhibiting effect of potassium iodide upon the development of the atherosclerosis produced by cholesterol was due to the potassium ion or to the iodine, and to rule out a non-

TABLE V

Effect of KBr upon Production of Atherosclerosis by Cholesterol

Rabbit No.	Sex	Weight		Duration of experiment	Cholesterol intake	KBr intake	Blood cholesterol	Atherosclerosis of aorta
		Start	End					
		kg.	*kg.*	*days*	*gm.*	*gm.*	*mg. per 100 cc.*	
A5-9	F.	1.4	1.6	100	39	39	424	Marked
A6-2	F.	1.2	1.7	100	39	39	297	0
A6-4	F.	1.3	1.7	100	39	39	431	Marked
A6-5	M.	1.5	1.6	100	39	39	431	Marked
A6-7	M.	1.8	2.1	100	39	39	325	Marked
A6-0	M.	1.6	1.2	101	39	39	293	Marked
A6-8	F.	1.6	1.2	101	39	39	798	Moderate
Average							428	

TABLE VI

Effect of KCl upon Production of Atherosclerosis by Cholesterol

Rabbit No.	Sex	Weight		Duration of experiment	Cholesterol intake	KCl intake	Blood cholesterol	Atherosclerosis of aorta
		Start	End					
		kg.	*kg.*	*days*	*gm.*	*gm.*	*mg. per 100 cc.*	
A7-6	M.	1.5	1.4	99	39	39	—	Moderate
A6-9	F.	2.0	2.0	101	39	39	511	Marked
A7-1	M.	1.6	1.9	101	39	39	490	Moderate
A7-2	F.	1.3	1.6	101	39	39	403	Marked
A7-3	F.	2.3	2.2	101	39	39	765	Marked
A7-4	F.	1.5	1.9	102	39	39	309	Moderate
A7-5	F.	1.4	1.9	102	39	39	544	Marked
A7-7	M.	1.6	1.8	102	39	39	119	0
A7-8	F.	1.6	1.8	102	39	39	284	Moderate
A8-9	M.	1.4	1.6	102	39	39	436	Moderate
Average							429	

specific halogen action, a group of rabbits was fed with cholesterol and potassium bromide and a second group was given cholesterol and potassium chloride.

The results obtained with potassium bromide feeding are shown in Table V.

Each rabbit received the usual gram of cholesterol three times a week together with a gram of KBr in aqueous solution. Of the 7 rabbits in the group, 6 developed gross atherosclerosis. These animals showed a blood cholesterol ranging from 293 to 798 mg. The seventh animal had a normal aorta, macroscopically and microscopically, although a hypercholesterolemia was also present. The average blood cholesterol for the group was 428 mg.

There were 10 rabbits in the group given cholesterol and potassium chloride (Table VI). Nine developed atherosclerosis. All had a hypercholesterolemia of from 284 to 765 mg. The one animal in this group that did not show atherosclerosis was the only one with a normal blood cholesterol. The average value for the cholesterol in the blood in the 9 rabbits in which it was determined, was 429 mg.

Discussion.—The effects of KBr, KCl, and KI upon the atherosclerosis produced in the aorta of rabbits by feeding cholesterol may be summarized as follows:

Group	No. of rabbits	Atherosclerosis		Average blood cholesterol
		Present	Absent	
				mg.
Cholesterol	3	3	0	608
Cholesterol + KBr	7	6	1	428
Cholesterol + KCl	10	9	1	429
Cholesterol + KI	12	1	11	183

The effectiveness of potassium iodide in preventing cholesterol atherosclerosis when administered simultaneously with the cholesterol is apparent. That this is due to the iodine and not to the potassium and that it is not a general halogen effect is suggested by the inadequacy of both the bromide and chloride.

Liebig (4) was the first to report a retarding influence of iodine upon cholesterol atherosclerosis in rabbits. Using an organic iodine preparation he at first was able to demonstrate no effect. Later, after increasing the amount of iodine administered, he was able to prevent the development of lesions in 3 of the 4 rabbits used. Subsequently (5) he stated that he had increased his series to 28 animals and that in 75 per cent he found either no lesion or only slight lesions in the aorta. No protocols were given.

Seel and Creuzberg (6) fed 3 rabbits cholesterol and egg yolk for 30 days. The serum cholesterol showed a prompt rise, with a fall to

normal within 3 weeks after cholesterol was stopped. The animals were killed later and it was stated that more or less atherosclerosis was found in the aortas. Three other rabbits were given cholesterol in the same manner for 30 days and then given an organic iodine preparation. There was no atherosclerosis when the rabbits were autopsied later. Finally, to 3 rabbits cholesterol and then potassium iodide were given. Aortic lesions were absent in 2, present in 1. Because of the importance of the time factor in the development of atheromatous lesions after cholesterol feeding, this work fails to be convincing, as 30 days seem too short a time to ensure the presence of aortic changes.

SUMMARY

1. Whole thyroid gland when administered simultaneously with cholesterol prevented the atheromatous changes produced by the latter in the aorta of rabbits in 17 of 19 animals.

2. In this series thyroxin was less effective, as atherosclerosis occurred in 8 of 11 rabbits.

3. Potassium iodide also exerted a strong protective action as aortic lesions were present in only 1 of a series of 12 rabbits fed cholesterol and potassium iodide concurrently.

4. The effectiveness of potassium iodide was not shared by potassium bromide or potassium chloride.

5. A relationship was noted between the level of the cholesterol in the blood and the development of atherosclerosis. In general, the aortic lesions accompanied a hypercholesterolemia.

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