FURTHER OBSERVATIONS ON THE BLOOD CHOLESTEROL OF RABBITS IN RELATION TO ATHEROSCLEROSIS*

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It was reported previously (1) that dried whole thyroid or potassium iodide administered concurrently with cholesterol to rabbits prevented the usual hypercholesterolemia and atherosclerosis. Thyroxin seemed less effective, and the bromide and chloride of potassium were ineffective. The protective action of the iodide was abolished by removal of the thyroids (2). Since these reports, further observations have been made on cholesterol-fed rabbits, the results of which are given herewith.

Rosenthal (3) found that rabbits fed cholesterol and small amounts of iodide actually developed a higher blood cholesterol and more marked atherosclerosis than those animals fed cholesterol alone. The amount of iodide given was calculated from a human therapeutic dose on the basis of comparative weights. It was pointed out quite rightly that, were the amount of iodide necessary to prevent hypercholesterolemia in rabbits translated proportionally into terms of human therapy, severe iodism would undoubtedly result. Rabbits, however, are notably tolerant to iodine, remaining in good condition even on huge doses. Nor does it seem with our present knowledge that results obtained in herbivores can be applied through inference to human beings or *vice versa*.

Page and Bernhard (4) also found that rabbits fed cholesterol and an organic iodide developed an average plasma cholesterol higher than those fed cholesterol alone. The animals given iodide, however, were largely protected from atherosclerosis.

The apparent discrepancy in the action of iodine upon the level of cholesterol in the blood has been explained to a large degree in an interesting report by Breusch and Thiersch (5). These investigators found that the effect of iodine depended upon the dose. Large doses of iodide entirely prevented a rise in the blood cholesterol while small amounts actually augmented this rise in cholesterol-fed rabbits.

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Methods

The rabbits used in this work were mostly of the Dutch belted variety. They were 4 to 6 months old when received unless otherwise noted. The animals were kept indoors in individual cages. The diet consisted of oats and fresh vegetables.

Each rabbit was given 1 gm. of crystalline cholesterol mixed with the grain three times a week.

Blood was obtained from the ear vein at 7 or 10 day intervals. Cholesterol determinations were made on the whole blood by the method of Bloor, Pelkan and Allen (6).

Duration of Protective Action of Potassium Iodide

Although potassium iodide was effective in preventing a rise in the blood cholesterol when fed in large amounts with cholesterol to rabbits

TABLE I

Duration of Protective Action of Potassium Iodide

Rabbit No.	Blood cholesterol, *mg. per 100 cc.*										
	Months										
	0	1	2	3	4	5	6	7	8	9	10
2-35	123	189	167	222	166	219	384	521	497	548	503
2-36	113	151	133	161	169	158	182	282	444	495	475
2-37	123	149	143	138	184	192	237	239	321	—	—
2-38	123	146	120	137	170	216	226	303	184	141	200
2-39	108	125	123	143	162	247	251	238	382	393	296
Average.....	118	152	137	160	170	206	256	317	366	394	369

(2) it was noted that, toward the end of the feeding period, which was arbitrarily set at 110 days, there was a tendency for the level of cholesterol in the blood to rise. This led to the conjecture that, possibly, the protective action of the iodide was impermanent and that, after a time, the animal might "escape" from the iodide effect resulting in the development of a hypercholesterolemia.

To test this possibility rabbits were fed cholesterol and potassium iodide over a period of 10 months.

Five rabbits were used. There were 3 males and 2 females. Cholesterol was given as described above. Each animal also received 1 gm. of potassium iodide

in aqueous solution three times a week mixed with the grain. Blood cholesterol determinations were made every 10 days.

Rabbit 2-37 died toward the end of the 8th month and was not autopsied. The other 4 animals remained in good health and were killed after 10 months. At autopsy all showed atheromatous changes in the aorta ranging from slight to marked in degree. In the gross there was an increase in liver fat in 3.

The blood cholesterol values are shown in Table I. For the sake of conciseness the average of the three monthly readings is given in each instance.

The iodide exerted a protective action and prevented a significant rise in the blood cholesterol for the first 4 months. Thereafter it seemed to lose its effectiveness, and the blood cholesterol mounted steadily throughout the remaining 6 months, much as it does in rabbits fed cholesterol alone.

This loss of effectiveness is curious and unexplained.

Effect of Potassium Iodide after Prolonged Cholesterol Feeding

Up to this point the work had been concerned with the effect of various substances on the blood cholesterol of rabbits when given at the beginning of the cholesterol feeding. It was now of interest to determine the effect of potassum iodide on the hypercholesterolemia of rabbits that had been fed cholesterol for a long time.

Nine rabbits were available that had been given cholesterol continuously for 11 months. Experience has shown that rabbits fed for as prolonged a period as this should be thoroughly "saturated" with cholesterol. If the animals were autopsied, marked atheromatous lesions in the arteries and extensive fatty deposits in the viscera presumably would be found.

The cholesterol feeding was continued. In addition, each animal was given a gram of potassium iodide thrice weekly for a month.

The blood cholesterol values are shown in Table II, and are expressed graphically in Fig. 1. The first five determinations given in the table were made during the 10th and 11th months of cholesterol administration and serve as a base line for comparison with the effect of the iodide. Essentially similar figures were obtained during previous months of feeding but have been omitted as irrelevant.

It is apparent that the administration of the potassium iodide produced a sharp rise in the already high blood cholesterol level. When the iodide was stopped the cholesterol values fell again, less abruptly than they had risen, and were approaching the former base line when

TABLE II

Effect of Potassium Iodide after Prolonged Cholesterol Feeding

Rabbit No.	Blood cholesterol, *mg. per 100 cc.*													
	10 day periods													
						Potassium iodide fed								
1-90	730	682	636	766	670	832	750	872	682	625	568	647	595	—
1-92	432	399	528	486	514	514	686	615	586	832	892	854	1042	—
1-94	647	658	682	636	780	736	961	1013	1102	782	720	781	750	646
1-95	421	338	389	361	425	507	586	560	698	638	487	577	568	500
2-01	475	568	568	528	529	721	708	1137	1102	782	735	593	—	—
2-02	353	463	413	379	350	329	568	658	503	577	395	452	470	521
2-04	239	310	298	291	293	310	335	357	338	347	417	500	458	—
2-05	446	357	264	395	417	441	658	—	708	765	625	514	368	436
2-06	399	289	318	248	268	290	521	—	544	576	586	354	399	371
Average.....	460	452	455	454	472	520	641	745	696	658	603	586	581	495

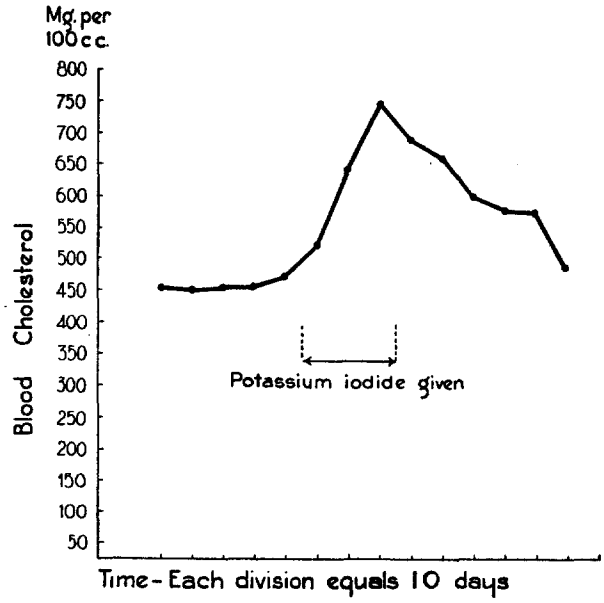

FIG. 1. Curve showing the sharp rise produced by potassium iodide in the average blood cholesterol values of a group of rabbits with long standing hypercholesterolemia.

an epidemic of "snuffles" killed 5 of the rabbits and ended the experiment.[1]

Effect of Thyroid after Prolonged Cholesterol Feeding

In view of the similar action of potassium iodide and whole thyroid in preventing a rise in blood cholesterol when given concurrently with cholesterol to rabbits (1), it was of interest to determine also the effect of thyroid upon the blood of these animals with long standing hypercholesterolemia.

TABLE III

Effect of Whole Thyroid on Long Standing Hypercholesterolemia

Rabbit No.	Blood cholesterol, *mg. per 100 cc.*							
	10 day periods							
				Thyroid fed				
1-89	463	472	431	278	618	705	862	862
1-93	457	581	704	438	506	676	820	736
1-94	452	506	486	359	355	589	463	526
2-02	647	603	556	263	388	446	495	428
2-06	551	376	413	236	284	505	834	1000
2-23	452	481	544	373	556	516	659	833
2-30	422	528	464	270	276	305	416	714
Average.....	492	507	514	317	426	535	650	729

A group of 7 rabbits was used. There were 5 males and 2 females. 4 animals had been fed cholesterol for 19 months and three had received it for 5 months. All had a markedly elevated blood cholesterol. 3 of the rabbits had been used several months previously to determine the effect of potassium iodide upon the hypercholesterolemia.

The cholesterol feeding was continued. Blood samples were taken every 10 days and the cholesterol determined. For a period of 3 weeks, dried whole thyroid was mixed with the grain to which cholesterol had been added. Each rabbit received approximately 0.4 gm. of the thyroid three times a week.

The results are shown in Table III and Fig. 2. The values in the second, third and fourth columns of the table represent a control period of a month before the

[1] The same effect has been observed recently in a 10th rabbit. The blood cholesterol in this animal tripled on potassium iodide, rising from 240 mg. to 821 mg., and dropping back to 213 mg. when the iodide was stopped.

thyroid was started. In the next two columns are the results obtained during the time that thyroid was fed. The last three columns list the determinations during the month after the thyroid feeding.

Contrary to the effect of potassium iodide, which produced a rise in the level of the hypercholesterolemia, thyroid caused a sharp fall. This decrease, however, was not sustained, and even before the thyroid administration was stopped the blood cholesterol began to rise toward

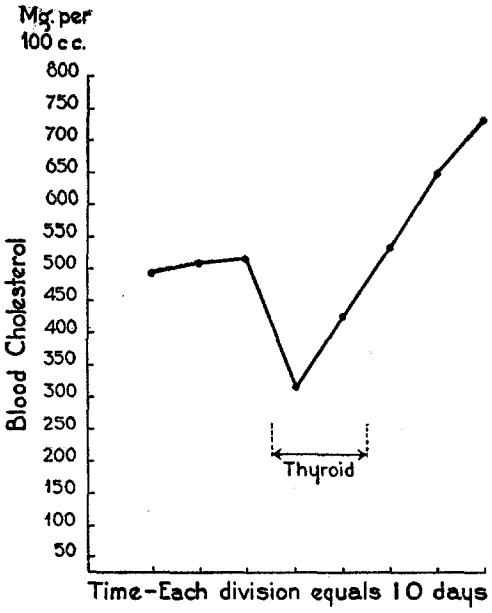

FIG. 2. The effect of whole thyroid upon the hypercholesterolemia of rabbits. After an immediate and marked drop in the blood cholesterol, it begins to rise even before the thyroid is discontinued and finally reaches a level higher than that in the preceding control period.

its previous level. Following the cessation of thyroid feeding the amount of cholesterol in the blood not only reached its former value but actually rose considerably higher than before. This is shown by the curve in Fig. 2.

Relative Effectiveness of Whole Thyroid and Thyroxin

In previous work (1) thyroxin had seemed somewhat less effective than whole thyroid in preventing a rise in the blood cholesterol of

rabbits fed cholesterol. In an attempt to amplify this finding a group of rabbits was subjected to a complete thyroidectomy. These animals, when fed cholesterol and potassium iodide, should exhibit a prompt rise in blood cholesterol, as the iodide is ineffective in preventing this in the absence of the thyroid (2). However, if whole thyroid or thyroxin were administered in an effort to substitute for the removed gland, the rise in blood cholesterol might be prevented. Furthermore, this procedure could throw some light upon the relative effectiveness of the two substances.

Thyroidectomized rabbits were divided into two groups, each containing 9 animals. Blood cholesterol determinations were made every 10 days. About a month was allowed after the operation to permit the animals to recover completely and to make sure that no spontaneous rise in blood cholesterol was to occur. Both groups were then started on cholesterol and potassium iodide given according to the usual procedure thrice weekly. In addition, each rabbit in the first group received 0.4 gm. of dried whole thyroid mixed with its grain three times a week. Each animal in the second group received 0.7 mg. of thyroxin by hypodermic injection twice a week. Thus the rabbits in the two groups received roughly equivalent dosage so far as thyroxin was concerned.

At the end of the feeding period of 110 days the surviving rabbits were killed. At autopsy the complete removal of the thyroid was verified and the aorta examined in the gross for atheromatous plaques.

The blood cholesterol determinations are given in Tables IV and V. Curves representing the average values are shown in Fig. 3.

The results were unsatisfactory. Examination of the tables reveals that the blood cholesterol of the individual rabbit was subject to considerable fluctuation but that, in most instances, there was a general tendency for it to rise. In other words, with the dosage used, it was impossible wholly to compensate for the removal of the thyroid by giving dried whole thyroid by mouth or thyroxin subcutaneously. It is fair to say, however, that the rise in blood cholesterol was less rapid than might be expected in a comparable group fed cholesterol alone.

Nor did the experiment show any noteworthy difference between the effectiveness of whole thyroid and thyroxin, although the averages of the former group were somewhat lower than the latter, as shown in Fig. 3. This difference is less convincing when it is observed that one of the animals (No. 1-64) in the group receiving thyroxin had a much

higher blood cholesterol than any of the others in the same group or, of more importance, in the group given whole thyroid. This necessarily resulted in a distortion of the averages.

TABLE IV

Thyroidectomized Rabbits Given Cholesterol, Potassium Iodide and Whole Thyroid

Rabbit No.	Blood cholesterol, *mg. per 100 cc.*											
	Days											
	1	10	20	30	40	50	60	70	80	90	100	110
1-61	135	90	104	139	121	158	170	138	182	200	200	184
1-79	103	94	134	109	144	188	194	163	164	127	148	229
2-07	132	127	151	175	191	252	197	226	253	284	395	229
2-08	128	116	120	130	211	271	163	276	250	280	291	303
2-09	127	139	182	169	203	242	211	284	308	264	347	347
2-10	124	138	112	133	134	136	184	166	223	162	253	189
2-11	123	112	146	135	185	137	150	159	156	156	268	221
2-12	108	102	123	112	123	129	126	129	107	107	140	108
2-13	108	199	243	241	278	202	166	159	155	114	164	142
Average..	121	124	146	149	177	191	173	189	200	188	245	217

TABLE V

Thyroidectomized Rabbits Given Cholesterol, Potassium Iodide and Thyroxin

Rabbit No.	Blood cholesterol, *mg. per 100 cc.*											
	Days											
	1	10	20	30	40	50	60	70	80	90	100	110
1-58	99	117	122	101	163	187	302	264	210	292	320	250
1-62	116	104	121	140	112	154	198	237	250	190	208	216
1-64	104	134	178	228	166	280	318	493	426	646	694	721
1-76	95	131	96	80	74	98	182	154	88	121	142	160
2-14	112	167	172	211	218	184	250	232	—	—	—	—
2-16	169	149	150	158	155	188	208	—	—	—	—	—
2-17	140	150	175	150	134	159	169	160	272	272	272	253
2-18	97	145	211	171	215	293	293	280	341	284	347	354
2-19	110	158	250	153	149	195	232	221	247	223	—	—
Average..	116	139	169	155	154	193	239	255	262	290	331	326

While the experiment was unsatisfactory in these respects it is perhaps important from another aspect. It is generally accepted that

a high blood cholesterol in a rabbit leads to the development of atheromatous plaques in the aorta. Although most of the rabbits in these two groups showed significant rises in blood cholesterol, it is noteworthy that at autopsy gross atherosclerosis of the aorta was present in only 1 animal of each group (Nos. 1-64, 2-09) and in each case the changes were minimal. This corroborates the findings of Page and Bernhard (4) who reported that rabbits given cholesterol and iodide developed a lipemia but that atherosclerosis was slight or absent at autopsy.

This finding becomes all the more perplexing when it is recalled that those rabbits that escaped from the protective action of large doses of

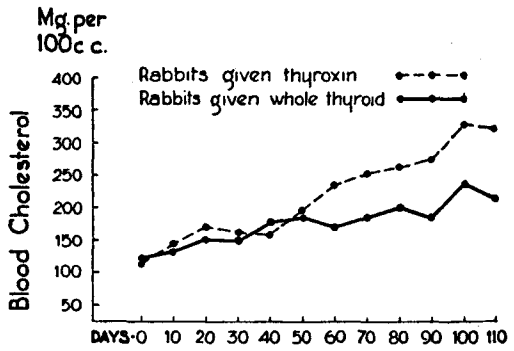

FIG. 3. The comparative effect of thyroid and thyroxin on the blood cholesterol of thyroidectomized rabbits fed cholesterol and potassium iodide.

iodide, as reported above, and developed a hypercholesterolemia all showed atherosclerosis at autopsy. Thus it seems very possible that iodide delays but does not ultimately prevent atherosclerosis in the presence of a high blood cholesterol. Perhaps if the rabbits in the present groups had been fed cholesterol over a longer period they would have shown atherosclerotic changes.

Effect of Age upon Response to Cholesterol Feeding

Individual rabbits differ markedly in the response of their blood cholesterol to cholesterol feeding. Although a rise usually occurs, this increase varies in degree. Cholesterol is distinctly an abnormal component of an herbivore's diet. In a sense, then, the less a rabbit's blood cholesterol rises when large amounts of cholesterol are added

to the diet, the more efficient that animal's organism may be said to be. The mechanism whereby the result is obtained is for the moment immaterial.

It seemed possible that an age factor might be present—*i.e.*, the older the rabbit, the less able its organism would be to adjust itself to the presence of an unusual dietary substance and the higher would be the resultant rise in blood cholesterol.

TABLE VI

Effect of Age on Development of Hypercholesterolemia

Rabbit No.	Blood cholesterol, *mg. per 100 cc.*																	
	Weeks																	
	0		1	2	3	4	5	6	7	8		9	10	11	12	13	14	15	16
Young Rabbits		Cholesterol started									Cholesterol stopped								
2-51	128		135	158	169	187	165	171	247	236		269	192	139	135	138	140	126	138
2-52	130		145	200	264	264	305	318	536	454		472	435	222	417	340	259	172	150
2-53	140		179	156	174	170	241	234	223	190		191	150	153	120	113	132	118	124
2-54	143		172	165	193	189	191	229	307	263		187	236	189	139	159	118	109	92
2-55	142		163	142	200	208	210	250	221	179		175	170	151	172	130	125	106	107
Average..	137		159	164	200	204	222	240	307	264		259	227	171	197	176	155	126	122
Old Rabbits																			
2-56	137		177	189	276	312	469	625	604	602		556	532	476	550	432	428	376	327
2-57	132		169	159	213	247	368	536	568	694		650	633	435	318	376	249	207	200
2-58	134		158	138	155	188	186	240	264	379		313	261	172	142	156	121	138	118
2-59	137		164	130	150	240	318	332	468	340		156	117	113	88	115	122	113	113
2-60	136		206	195	293	329	493	536	658	640		618	458	362	316	355	424	379	363
Average..	135		175	162	217	263	367	454	512	531		458	400	312	283	287	269	243	224

To test this thesis, the results of cholesterol feeding upon the blood cholesterol of two groups of rabbits—one young, the other old—was observed. There were 5 animals in each group, and in each case there were 4 females and 1 male. The rabbits in the first group were 4 to 5 months old at the beginning of the experiment; the age of those in the second group ranged from 2 to 3 years. The feeding of cholesterol was begun simultaneously for both groups and was carried out by the usual method.

The results are shown in Table VI and Fig. 4. During the cholesterol feeding the blood cholesterol of the older rabbits rose faster and to much higher levels than that of the young rabbits. When the feeding was stopped the resulting fall in blood cholesterol was more gradual and protracted in the older group. Admittedly the number of rabbits was small and no sweeping conclusions may be drawn. Nevertheless, the difference in response of the two groups was striking.

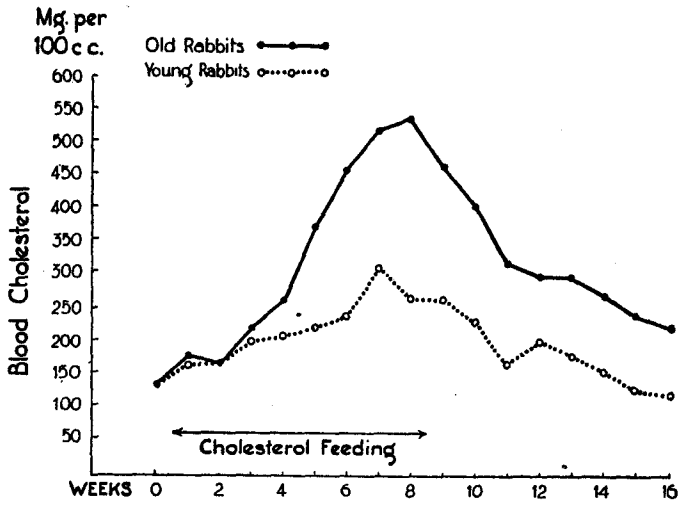

FIG. 4. Curves comparing the results of cholesterol feeding on the blood cholesterol of young and old rabbits. The rise in the latter is more abrupt and more marked; the corresponding fall is more gradual and prolonged.

SUMMARY

1. The action of potassium iodide in preventing a significant rise in the blood cholesterol of rabbits fed cholesterol was temporary. After about 4 months it lost its effectiveness and the blood cholesterol rose.

2. In rabbits with hypercholesterolemia resulting from long continued cholesterol feeding, the administration of potassium iodide caused a marked rise in the blood cholesterol.

3. On the other hand, dried whole thyroid given to such animals produced a sharp fall in the blood cholesterol. This fall was temporary and was followed by a rise to new high levels.

4. In thyroidectomized rabbits fed cholesterol and potassium iodide,

both thyroid and thyroxin delayed but did not prevent a rise in blood cholesterol. Even with the hypercholesterolemia in these animals, however, the incidence of atherosclerosis was low.

5. Age apparently played a part in determining the response of the blood cholesterol to cholesterol feeding. In a group of old rabbits when compared with a younger group the rise in the cholesterol of the blood was greater and the subsequent return toward normal was slower when the feeding was stopped.

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