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DISCLAIMER

EFFECT OF INOSITOL AND OCTADECYL ALCOHOL FEEDING ON LIPOPROTEINS IN CHOLESTEROL FED RABBITS*

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INTRODUCTION

A controversy exists as to the effect of inositol and other lipotropic agents on serum cholesterol levels and atherosclerosis in humans and animals. There is no general agreement that these agents lower serum cholesterol levels or exert an inhibitory effect on the development of atherosclerosis[1]. Dotti, Felch and Ilka[2] report an inhibition by inositol of the expected rise in serum phospholipids and cholesterol following cholesterol feeding in the rabbit. On the other hand Broun et al[3]. Ellenbogen and Kendall[4], and Moses[5] find no effect of inositol on serum lipids following cholesterol feeding in rabbits. These latter authors also report no influence of inositol on the development of atherosclerosis in the cholesterol fed rabbit.

Gofman et al. have described a spectrum of lipoproteins in the serum of humans and animals[6,7,8]. The lipoproteins are described using flotation rates (S_f) in the ultracentrifuge and the spectrum of lipoproteins found in human and animal sera includes lipoprotein species from S_f 2 up to S_f 40,000 (S_f 40,000 represents chylomicrons). The spectrum of lipoproteins accounts for all the lipids found in serum, and any reduction or increase in serum lipids is reflected in a reduction or increase in one or many species of lipoproteins.

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Consequently any effect of inositol on the cholesterol and phospholipid levels of the cholesterol fed rabbit must be reflected by an alteration in all or a part of the lipoprotein spectrum.

One class of lipoproteins, the S_f 12-20 class, correlates well with atherosclerosis in the rabbit[9]. In the human, two classes of lipoproteins, the S_f 35-100 class, correlate well with atherosclerosis[10]. There is some residual correlation between atherosclerosis and lipoprotein classes from S_f 20 to S_f 100 in the rabbit and it is doubtful if any species difference exists between rabbits and humans[9].

The normal rabbit fed cholesterol rapidly develops large quantities of all classes of lipoproteins. After large quantities of lipoproteins have appeared in the serum following cholesterol feeding, they gradually disappear following cholesterol withdrawal.

In view of the above considerations, the effect of inositol on the rate of development of various classes of lipoproteins during cholesterol feeding in the rabbit was investigated. Experiments were performed to determine the rate of disappearance of lipoproteins of various classes following cholesterol withdrawal, and to determine the effect of inositol on this rate of disappearance.

The experiments described herein were undertaken as "pilot" experiments to give a general insight into the problems involved in carrying out such feeding studies. It would be necessary to extend this work with a large number of animals before any definite conclusions could be inferred from the results.

METHODS

Female rabbits of the New Zealand White Strain, weighing between 2.5 and 3.0 kg, were used in all experiments. One gram of cholesterol was dissolved in 5 cc. of Wesson Oil by gentle heating, and mixed with 100 gms of Albers Family Style Rabbit Pellets. The rabbits were given this food and water ad libitum

during the cholesterol feeding experiments. Inositol was added to this food or to non-cholesterolized rabbit pellets to make a 1.0 gm percent mixture during inositol feeding procedures.

Two rabbits were fed cholesterol and inositol containing food for 28 days (Group A), and 2 rabbits received cholesterol containing food only for 28 days (Group B). At the end of this time the 2 rabbits comprising Group A were sacrificed and the degree of aortic atherosclerosis determined.

The two rabbits fed cholesterol food only for 28 days (Group B) were put on inositol containing food at this time and cholesterol was withdrawn. After 42 days (70 days from the start of the experiment) they were sacrificed and the degree of aortic atherosclerosis determined.

Blood specimens were obtained at frequent intervals as indicated in Tables I and II, and analyzed for the S_f 5-15, 15-30, 30-100 and 100-400 classes of lipoproteins using the ultracentrifugal technique of Gofman et al[6].

RESULTS

Tables I and II summarize the changes in lipoproteins found in the feeding regimen used. The changes found in feeding cholesterol and inositol simultaneously are shown in Table I, and the effects of feeding cholesterol alone are shown in the figures up to Day 28 in Table II. Table II also shows the changes in lipoprotein classes found after stopping cholesterol and starting inositol feeding. It can be seen that control levels of lipoproteins were reached by Day 70 in both rabbits receiving inositol (Group B).

There is no significant difference between the rate of development of the various lipoprotein classes between the animals fed both cholesterol and inositol and cholesterol alone. Data from a large series of rabbits fed cholesterol shows a similar rate of development of lipoproteins in the serum[9], and there is no evidence in the two animals fed inositol and cholesterol that any retarding

effect on the appearance of lipoproteins can be ascribed to inositol.

Data presented in Table III show the rate of disappearance of serum lipoproteins following cholesterol withdrawal. These data are representative of results obtained in this laboratory using a large number of animals[11].

There is no significant difference in the rate of disappearance of lipoproteins following cholesterol withdrawal in rabbits fed inositol when compared with feeding plain food.

At the end of the feeding regimens, the animals were sacrificed by air embolism. The thoracic aortas were removed, and atherosclerosis graded macroscopically on a scale of 1+ to 5+, with 5+ being most severe atherosclerotic disease. The two animals fed cholesterol and inositol (Group A) showed 3+ and 4+ aortic disease. This amount of atherosclerosis is within the normal limits following cholesterol feeding for 28 days. The two animals maintained on inositol following cholesterol withdrawal (Group B) both showed 3+ atherosclerotic disease. This amount of atherosclerosis is within the normal limits following a 42 day period of cholesterol withdrawal subsequent to a 28 day period of cholesterol feeding.

It has been reported[12] that simultaneous feeding of cholesterol and ethyl alcohol results in lowering of serum cholesterol levels. In view of the fact that ethyl alcohol may impair liver function which might lead to such lowering[13], it was considered of interest to determine the effect of a higher primary alcohol when fed together with cholesterol. For this experiment octadecyl alcohol was selected for assay as to its effect on serum lipoproteins with and without cholesterol feeding in the rabbit. Octadecyl alcohol was dissolved in ether and poured over rabbit pellets to make a 1 percent containing mixture, and the ether evaporated. The rabbits were maintained on this diet with or without cholesterol food for two weeks. One normal rabbit (Rabbit 7) was fed octadecyl alcohol (1 percent) on rabbit pellets alone; one normal rabbit (Rabbit 8) was fed octadecyl alcohol (1 percent) and cholesterol (1 percent) food (no Wesson Oil); and

a third rabbit (Rabbit 9), which had been fed cholesterol food (no Wesson Oil) for 2 months, was started on 1 percent octadecyl alcohol and 1 percent cholesterol containing food.

In the normal rabbit fed octadecyl alcohol, no lipoprotein changes were observed. In the rabbit fed cholesterol and octadecyl alcohol, there was a rapid development of all classes of lipoproteins developing at a rate not significantly different from that observed with the cholesterol - Wesson Oil diet. The rabbit fed cholesterol for 2 months showed changes in serum lipoproteins after the start of octadecyl alcohol and cholesterol food similar to those observed upon initiation of Wesson Oil feeding. These data are presented in Table IV. It has been observed in this laboratory that Wesson Oil, or other fat, accelerates the rate of development of serum lipoproteins following cholesterol feeding in rabbits. Stetten and Schoenheimer[14] have pointed out that deutero-octadecyl alcohol is converted to deuterostearic acid in the gut, and this fact might explain the findings observed with octadecyl alochol feeding in these experiments.

CONCLUSIONS

1. Two rabbits fed inositol and cholesterol developed all classes of serum lipoproteins at the same rate as controls fed cholesterol only.

2. Two rabbits who had developed large quantities of all classes of lipoproteins following cholesterol feeding were taken off cholesterol food and put on inositol containing food. The rate of disappearance of lipoproteins from the serum of rabbits on a diet of inositol plus normal food was no different from the rate of disappearance of these lipoproteins from the serum of cholesterol pre-fed rabbits maintained on normal food alone.

3. Octadecyl alcohol feeding was without effect on the lipoproteins of a

normal rabbit. In a rabbit simultaneously fed cholesterol and octadecyl alcohol there was a rapid development of all classes of lipoproteins. In a rabbit fed cholesterol for 2 months prior to octadecyl alcohol feeding there was a rapid increase in all classes of lipoproteins following feeding of the alcohol. These results are similar to those obtained with any fat.

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TABLE I

Effect of cholesterol plus inositol feeding on serum lipoproteins in rabbits (Group A).

		Serum Lipoproteins, mg. %			
Rabbit	Day	S_f 5-15	S_f 15-30	S_f 30-100	S_f 100-400
1	0	55	7	9	2
	1	165	73	44	4
	2	242	70	22	0
	3	308	154	88	7
	7	925	349	220	150
	10	842	759	550	473
	14	941	1139	693	726
	28	143	935	1397	1166
2	0	46	2	0	0
	1	77	4	0	4
	2	195	11	4	0
	3	316	44	48	7
	7	679	92	29	0
	10	666	325	204	264
	14	781	605	209	281
	28	138	468	407	331

TABLE II

Effect of feeding cholesterol alone (28 days); then inositol alone (additional 42 days) on serum lipoproteins in rabbits. (Group B)

Rabbit	Day	Serum Lipoproteins, mg. %. S_f5-15	S_f15-30	S_f30-100	S_f100-400
3	0	239	36	6	0
	1	121	7	4	0
	2	88	29	15	0
	3	51	18	37	0
	7	165	117	114	7
	28	1133	781	451	451
	32	479	891	237	17
	35	803	479	72	0
	42	477	73	4	0
	49	501	116	28	3
	56	343	31	9	2
	70	84	18	20	13
4	0	77	18	7	4
	1	55	7	18	0
	2	169	44	4	0
	3	121	62	165	84
	7	198	11	18	0
	28	165	842	468	94
	32	182	567	402	99
	35	281	154	171	6
	42	172	18	4	0
	49	77	19	17	3
	56	53	0	7	0
	70	26	11	0	0

TABLE III

Effect of cholesterol withdrawal on serum lipoproteins following 28 day cholesterol feeding.

Serum Lipoproteins, mg. %

Rabbit	Day	S_f5-15	S_f15-30	S_f30-100	S_f100-400
5	0	154	198	132	0
	7	68	26	35	2
	14	11	3	8	0
	21	24	15	35	4
	28	8	6	83	25
6	0	556	176	99	11
	7	264	95	123	7
	14	185	31	15	0
	21	141	20	24	2
	28	112	20	35	2
	35	128	11	20	4
	42	143	29	29	0

TABLE IV

Effect of octadecyl alcohol feeding on serum lipoproteins and cholesterol of rabbits. Rabbit 7 fed octadecyle alcohol only; rabbit 8 fed octadecyle alcohol plus cholesterol; rabbit 9 prefed cholesterol, fed octadecyl alcohol plus cholesterol.

	Serum Cholesterol (mg%)				Serum Lipoproteins (mg%)							
	Day 0		Day 14		Day 0				Day 14			
Rabbit	F	T	F	T	Sf 5-15	Sf 15-30	Sf 30-100	Sf 100-400	Sf 5-15	Sf 15-30	Sf 30-100	Sf 100-400
7	3	31	0	28	26	0	0	0	18	7	15	9
8	9	48	191	646	46	15	35	18	847	585	187	156
9	61	220	102	258	446	39	33	11	270	677	319	215

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