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The influence of reticuloendothelial stimulation on the response of rats to dietary cholesterol*

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SUMMARY

The influence of zymosan, and of various chemical fractions obtained from the yeast cell wall, on lipid alterations induced by the feeding of a cholesterol-cholate diet to rats was studied. Intravenous administration of zymosan produced pronounced stimulation and hyperplasia of the reticuloendothelial system, and a reduction in hepatic and plasma accumulation of ester cholesterol. Oral administration of zymosan was ineffective. Glucan, and other fractions derived from zymosan that stimulated the reticuloendothelial system, significantly inhibited dietary-induced hepatic cholesterosis. The results are indicative of a relationship between the functional activity of the reticuloendothelial system and cholesterol metabolism.

Previous studies (1–3) have demonstrated that the intravenous injection of zymosan produces marked hyperplasia and hyperfunction of the reticuloendothelial (RE) system. Zymosan administration to rats maintained on an atherogenic thrombo-embolic diet significantly decreased the plasma and liver accumulation of ester cholesterol (3–5). Based upon these and other observations (6–8), it was postulated that the relative hypocholesterolemic effect of zymosan was a reflection of the participation of Kupffer cells in cholesterol metabolism.

Subsequent studies indicated that glucan, a neutral polysaccharide, was an active RE-stimulating fraction in zymosan (9). In an attempt to evaluate the relationship of RE function to cholesterol metabolism, a study was undertaken of the effectiveness of zymosan, administered either orally or intravenously, in modifying the development of dietary cholesterosis and hypercholesterolemia. The influence of glucan and other chemical fractions derived from zymosan was also evaluated to determine the relationship between the ability of these agents to stimulate the RE system and to exert a hypocholesterolemic effect in rats fed a high-cholesterol, high-fat diet.

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METHODS

Male rats (Holtzman Co., Madison, Wis.), previously maintained on Purina chow, were fed a Purina chow diet containing 1% cholesterol and 0.5% sodium cholate. Zymosan[1] was administered intravenously in the amount of 0.7 mg/100 g for six days, beginning one day prior to the feeding of cholesterol. Control rats received equivalent volumes of isotonic saline, the suspending medium for the zymosan. When employed orally, zymosan was given in the amount of 10 mg/day for five days.

Glucan, prepared from zymosan by the method of Hassid (10), was employed either at a dose of 2.5 mg/100 g or 1 mg/100 g. The rats were injected intravenously daily for seven days and maintained on the cholesterol-cholate diet for the last six days.

A suspension of 75 mg of cholesterol in 1.5 ml of corn oil was administered by stomach tube twice daily for four days to other saline or glucan injected rats.

Plasma and liver lipids were extracted and purified by the Folch procedure as outlined by Sperry (11). Phospholipid phosphorus was determined by the method of King (12) and phospholipid was calculated as lipid P × 25. Total and free cholesterol were determined by the Sperry-Webb procedure (13).

[1] Zymosan was generously furnished by Standard Brands, Inc., New York City.

TABLE 1. INFLUENCE OF ZYMOSAN ON LIVER AND PLASMA LIPIDS* of RATS FED CHOLESTEROL CHOLATE

Group and Number	Liver						Plasma		
	Weight (g)	Percentage of Body Weight	Phospholipid	Cholesterol		Neutral Fat	Phospholipid	Cholesterol	
				Free	Ester			Free	Ester
Normal diet + saline (11)	9.90 ± 0.82	3.5 ± 0.1	38.0 ± 0.9	2.23 ± 0.07	0.24 ± 0.06	9.52 ± 0.97	118 ± 6	14.5 ± 1.7	47 ± 2
Cholesterol-fed + saline (8)	11.96 ± 0.52	4.7 ± 0.3	32.5 ± 0.7	3.11 ± 0.09	22.28 ± 2.33	14.60 ± 1.27	152 ± 5	41.3 ± 3.3	195 ± 13
Cholesterol-fed + zymosan (7)	13.61 ± 0.44	4.8 ± 0.2	31.8 ± 0.7	2.95 ± 0.07	12.54 ± 2.68	14.92 ± 1.00	127 ± 5	30.3 ± 3.6	123 ± 11

* Lipid values are expressed as mg per g of fresh tissue or per 100 ml of plasma ± standard error of mean.

Neutral fat was originally calculated by difference and subsequently glycerides were determined on the chloroform–methanol extract by the method of Van Handel and Zilversmit (14).

All data were statistically analyzed at the 95% confidence level either by the analysis of variance or by the "*t*" test for the difference between means.

RESULTS

The administration of the cholesterol-cholate diet for five days to normal saline-injected male rats was associated with a significant reduction in the concentration of liver phospholipid, and an increase in free and ester cholesterol and neutral fat (Table 1). The influence of zymosan administration on various lipid fractions of plasma, liver, lung, and spleen of normal rats as well as those maintained on an "infarct-producing" diet has been reported (3, 5).

The intravenous administration of zymosan to rats maintained on the cholesterol-cholate diet was associated with a 45% reduction in the concentration of liver ester cholesterol. In contrast, no change was observed in either the phospholipid, free cholesterol, or neutral fat fractions. Liver weight, which was significantly increased above normal in the cholesterol-fed, saline-injected group, was not significantly increased by zymosan administration.

The plasma free and ester cholesterol levels were increased approximately three- and four-fold respectively in the cholesterol-fed, saline-injected rats and plasma phospholipid concentration was also significantly increased. In contrast, a significantly smaller accumulation of plasma phospholipid and ester cholesterol was observed in the zymosan-treated group. The initial and final body weights as well as hematocrits in the various groups did not differ significantly.

In contrast to the effectiveness of zymosan administered intravenously in inhibiting plasma and hepatic ester cholesterol accumulation, the oral administration of zymosan was ineffective in rats maintained on the cholesterol diet (Table 2). The oral administration of zymosan did not result in any increase in weight of liver, lung, or spleen, the latter a most sensitive sign of RE stimulation induced by intravenously administered zymosan.

During the study that resulted in the identification of glucan as the active agent in zymosan, several fractions were obtained from zymosan that had RE-stimulatory activity and a lipid fraction was isolated that had no influence on phagocytic function (9). These various fractions were administered daily for six days in the

TABLE 2. FAILURE OF ORALLY ADMINISTERED ZYMOSAN TO MODIFY PLASMA AND LIVER LIPIDS*

Group	Liver						Plasma			
	Weight (g)	Percentage of Body Weight	Phospholipid	Cholesterol		Neutral Fat	Phospholipid	Cholesterol		Neutral Fat
				Free	Ester			Free	Ester	
Cholesterol-fed	11.36 ± 0.56	4.8 ± 0.2	36.4 ± 0.3	3.00 ± 0.18	20.25 ± 1.87	14.24 ± 1.11	155 ± 9	48.9 ± 6.6	194 ± 25	175 ± 38
Cholesterol-fed + oral zymosan	11.92 ± 0.51	4.8 ± 0.2	35.1 ± 0.7	2.86 ± 0.18	22.22 ± 1.53	17.10 ± 1.71	173 ± 8	51.2 ± 4.8	228 ± 14	149 ± 31

* Lipid values, expressed as mg per g of fresh liver or per 100 ml of plasma ± standard error of the mean, are derived from 6 rats in a group.

TABLE 3. INFLUENCE OF VARIOUS ZYMOSAN FRACTIONS ON LIVER AND PLASMA CHOLESTEROL*

Fraction Injected	Body Weight (g)		Liver			Hematocrit	Plasma Cholesterol (mg/100 ml)	
			Weight (g)	Cholesterol (mg/g)				
	Initial	Final		Free	Ester		Free	Ester
Tween-80 (Control)	256 ± 11	242 ± 7	9.05 ± 0.47	3.64 ± 0.04	26.37 ± 1.88	51 ± 2	28.0 ± 4.0	153 ± 26
Lipid I	252 ± 7	237 ± 8	9.29 ± 0.46	3.58 ± 0.12	22.02 ± 1.30	52 ± 1	30.1 ± 3.9	167 ± 19
Residue A	253 ± 9	228 ± 7	10.36 ± 0.35	3.44 ± 0.25	7.86 ± 1.76	50 ± 1	23.7 ± 2.7	103 ± 4
Residue B	246 ± 9	227 ± 8	9.71 ± 0.58	3.58 ± 0.10	17.89 ± 0.96	48 ± 1	27.9 ± 3.0	128 ± 11

* Values are means ± standard error compiled from 8 rats per group maintained on the cholesterol-cholate diet. Lipid I was a chloroform-ether soluble extract of zymosan. The residue remaining after removal of the readily extractable lipid (Lipid I) is called Residue A. Residue B was lipid-free zymosan. Lipid I had no RE-stimulatory activity, whereas Residues A and B induced RE hyperplasia and hyperfunction (9).

TABLE 4. INFLUENCE OF GLUCAN ON LIVER LIPID CONCENTRATIONS OF PAIR-FED RATS MAINTAINED ON 1% CHOLESTEROL, 0.5% SODIUM CHOLATE

Group and Number	Body Weight (g)		Organ Weight (g)			Liver Lipids*		
						Cholesterol		
	Initial	Final	Lung	Spleen	Liver	Free	Ester	Triglyceride
Saline (6)	310 ± 13	283 ± 10	1.44 ± 0.06	0.63 ± 0.05	9.62 ± 0.43	2.76 ± 0.06	12.21 ± 1.03	12.51 ± 0.39
Glucan (6)	311 ± 9	280 ± 11	4.19 ± 0.08	2.45 ± 0.21	14.72 ± 0.87	2.98 ± 0.08	3.28 ± 0.48	7.27 ± 0.90

* Values are expressed as mg of lipid per g of fresh liver ± standard error of mean.

amount of 1 mg/100 g suspended in 0.05% Tween 80 (polyoxyethylene sorbitan monooleate) to male rats maintained on the cholesterol-cholate diet for five days (Table 3). Control rats were injected with an equivalent volume (0.5 ml) of 0.05% Tween 80 and were also maintained on the cholesterol-cholate diet.

In agreement with the data presented in Tables 1 and 2, significant elevations from normal values occurred in the plasma and liver ester cholesterol fraction of rats maintained on the cholesterol-cholate diet. The administration of Lipid I, a fraction that does not stimulate the RE system, produced no effect on the accumulation of liver and plasma cholesterol. In contrast, a protein-polysaccharide complex (designated as Residue A), which was stimulatory to the RE system and induced hyperplasia (9), produced a significant reduction in liver and plasma ester cholesterol. Residue B, an active polysaccharide fraction (9), also produced significant decreases in liver and plasma ester cholesterol concentration. However, the reduction in liver ester cholesterol was only 32% in the group receiving Residue B, in contrast to the 70% reduction observed in the Residue A group.

The influence of glucan on liver weight and hepatic cholesterol and triglyceride concentrations of pair-fed rats maintained on the cholesterol-cholate diet for six days is presented in Table 4. The daily intravenous administration of glucan, initiated one day prior to cholesterol feeding, produced significant increases in liver, lung, and spleen weights. The liver ester cholesterol fraction, which was significantly increased above normal values in the cholesterol-fed group, was decreased 70% by simultaneous treatment with glucan (Table 4). The total liver ester cholesterol (liver weight × concentration) was 117 mg in the saline group in contrast to 48 mg in the glucan rats. The total liver content of free cholesterol was increased from 26.5 mg in the control group to 43.8 mg in the glucan-treated group. The liver content of total cholesterol (free + ester), however, was reduced 34%.

In order to evaluate the effectiveness of glucan in inhibiting hepatic cholesterosis in the presence of excess dietary triglyceride, rats were given a suspension of 75 mg cholesterol in 1.5 ml corn oil by oral intubation twice daily for a 4-day period. Glucan was injected in the amount of 1 mg/100 g and, as usual, injections were

TABLE 5. INFLUENCE OF GLUCAN ON LIVER LIPIDS OF CHOLESTEROL AND CORN OIL GAVAGED RATS

Group (Number)	Body Weight (g)		Organ Weight (g)			Cholesterol*		Phospholipid*	Triglyceride*
	Initial	Final	Liver	Lung	Spleen	Free	Ester		
Saline (4)	239 ± 3	232 ± 3	8.49 ± 0.42	1.66 ± 0.08	0.60 ± 0.01	2.40 ± 0.10	5.89 ± 0.86	32.1 ± 1.0	37.1 ± 9.3
Glucan (6)	243 ± 6	231 ± 5	12.23 ± 0.44	2.45 ± 0.20	1.58 ± 0.15	2.53 ± 0.08	1.45 ± 0.31	29.9 ± 0.5	28.8 ± 2.6

* Lipid values are expressed as mg of lipid per g of fresh liver ± standard error.

initiated 1 day prior to the administration of the cholesterol suspension so as to have a hyperactive RE system before the animal received the excess dietary lipids.

The liver phospholipid concentration (Table 5) was again significantly reduced in both the saline and glucan cholesterol-fed groups from the value of 38.0 mg/g noted in normal rats (Table 1). The liver triglyceride concentration was significantly increased above normal values in both groups. The free cholesterol concentration remained unaltered while the ester cholesterol fraction, which was increased in the saline-injected rats fed cholesterol and corn oil, was significantly reduced by the administration of glucan.

The total liver content of ester cholesterol (liver weight × concentration) in the saline-injected group was 50 mg in contrast to the 17.8 mg noted in the glucan group. The total liver content of free cholesterol was increased from 20 mg in the control to 31 mg in the glucan group. The total liver phospholipid was also increased in the glucan group from 271 mg to 365 mg. The phospholipid/free cholesterol ratio remained unaltered, suggesting the possible employment of these two lipid fractions in the structural organization of the newly formed Kupffer cells. The total liver cholesterol content (free + ester), however, was reduced 30% by glucan administration. Although the liver triglyceride concentration was significantly reduced by glucan, total liver triglyceride content was unaltered, demonstrating again the specificity of RE-stimulating agents in reducing primarily the ester cholesterol fraction (3–5).

DISCUSSION

The present studies reconfirm and extend previous observations of the inhibition of diet-induced hypercholesterolemia and hepatic cholesterosis in the rat by the intravenous administration of zymosan (3–5). The ineffectiveness of oral zymosan in modifying the development of hypercholesterolemia, due to possible lack of absorption, was also associated with its failure to induce RE hyperfunction and hyperplasia.

A study of the various fractions derived from zymosan manifesting an ability to stimulate the RE system revealed that they were capable of producing a significant inhibition of hepatic ester cholesterol accumulation. The reduction in accumulation of liver ester cholesterol occurred in both the presence (Tables 4, 5) and absence (Tables 1, 3) of liver hyperplasia, suggesting the hyperfunctional state to be a major factor. Conversely, a fraction obtained from zymosan that was inactive in respect to RE stimulation was ineffective in inhibiting the accumulation of hepatic and plasma cholesterol.

Glucan, a neutral polysaccharide, has been identified as the fraction in zymosan possessing the ability to induce RE cell hyperfunction and proliferation in mice and rats leading to hyperplasia of those organs containing RE elements (9). The hyperfunctional state induced by zymosan (1, 2, 9) and glucan (9), as determined by phagocytic function, is initially the result of an increased functional activity of pre-existing Kupffer cells and later the formation of new Kupffer cells. The prolonged administration of zymosan has been associated with an anemia that develops due to increased phagocytosis of erythrocytes. This anemia is reversible in nature (15). The measurement of the incorporation of tritiated thymidine has indicated an exclusive effect of zymosan on the proliferation of hepatic littoral or endothelial cells (2). The evaluation of hepatic function by sulfobromophthalein sodium (BSP) removal, an index of parenchymal cell activity, indicated normal BSP removal in animals with hyperfunctional RE systems (16). These studies indicate a rather exclusive influence of zymosan and glucan on Kupffer cells, and support the concept that the decreased hepatic cholesterol accumulation in zymosan- and glucan-treated rats fed cholesterol reflects the possible participation of these hyperfunctional RE cells in some as yet undefined aspect of cholesterol metabolism. In support of this concept, clinical

evidence has also been presented specifically relating a hyperactive RE system to the low serum cholesterol levels seen in many leukemic patients (17).

The participation of RE cells in cholesterol metabolism has been previously suggested. Kupffer cells have been demonstrated to accumulate cholesterol following the intravenous injection of hypercholesterolemic serum (18). The biotransformation of cholesterol has also been related to the activity of RE cells since macrophages have been demonstrated to show histochemical changes following uptake of cholesterol (7). The formation of cholesterol by Kupffer cells has been demonstrated to be comparable to its production by parenchymal cells (20) and the possible formation of lipoproteins by RE cell has been proposed (8, 19, 20). Cells comprising the RE system have also been demonstrated to be capable of esterifying cholesterol as well as hydrolyzing cholesterol esters (19). The involvement of the RE system in other areas of lipid metabolism is suggested since it has been reported that macrophages can hydrolyze and oxidize triglyceride and fatty acids (21). This observation may well explain the decreased accumulation of hepatic triglycerides noted in rats with hyperfunctional RE systems following carbon tetrachloride intoxication (22).

While the mechanism and degree of participation of RE cells in lipid metabolism and possibly atherosclerosis (3, 23) is not yet fully understood, it is obvious that the technique of selectively inducing a hyperfunctional state of the RE system by the neutral polysaccharide, glucan, will allow further evaluation of RE function in relation to various parameters of lipid metabolism.

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