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Effects of Gum Guar, Locust Bean Gum and Carrageenan on Liver Cholesterol of Cholesterol-Fed Rats.* (27585)

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Our previous studies(1) indicate that pectin N.F. when fed at a 5% level in the diet largely counteracted the increment in liver cholesterol and liver total lipids induced by cholesterol feeding in the rat. Other roughage or bulk-forming materials such as cellulose, agar, sodium alginate, protopectin and calcium silicate (Micro-Cel) were ineffective in this regard. Data are presented here indicating that Gum Guar, Locust Bean Gum and carrageenan also have significant activity in counteracting the increment in liver cholesterol and liver total lipids induced by cholesterol feeding in the rat.

Procedure. The basal ration consisted of sucrose, 61%; casein,† 24%; cottonseed oil, 10%; salt mixture,‡ 5%; and the following vitamins per kg of diet: thiamine hydrochloride, 20 mg; riboflavin, 20 mg; pyridoxine hydrochloride, 20 mg; calcium pantothenate, 60 mg; nicotinic acid, 100 mg; ascorbic acid, 200 mg; biotin, 4 mg; folic acid, 10 mg; p-aminobenzoic acid, 400 mg; inositol, 800 mg; 2-methyl-1,4 naphthoquinone, 5 mg; vit B_{12}, 150 μg; choline chloride, 2 g; vit A, 5000 U.S.P. units; vit D_2, 500 U.S.P. units; and alpha-tocopheryl acetate, 100 mg. Tests were conducted with rats fed the basal ration, the basal ration + 1% cholesterol, and the basal ration + 1% cholesterol + the various supplements indicated in Table I. The cholesterol and test supplements were incorporated in the basal ration in place of an equal amount of sucrose. Fifty-six male rats of the Holtzman strain with an average body wt of 43.6 g (range 38 to 50 g) were divided into 7 comparable groups of 8 each, placed in metal cages with raised screen bottoms (2 or 3 rats per cage), and provided the test diets and water *ad libitum*. Animals were fed on alternate days and all food not consumed 48 hours after feeding was discarded. Body weights were recorded weekly. After 28 days of feeding, the rats were anesthetized with sodium pentobarbital, and blood was withdrawn from the aorta into a heparinized syringe. Livers were excised, blotted to remove excess blood, weighed and stored in a freezer until analyzed. Lipid was extracted from the livers by the method of Thompson *et al.*(2), and cholesterol was determined on liver and plasma by the method of Nieft and Deuel(3). Total lipids were determined gravimetrically on an aliquot of the liver extract.

Results. The increment in liver cholesterol and liver total lipid induced by cholesterol feeding in the rat was largely counteracted by the concurrent feeding of Gum Guar, Locust Bean Gum or carrageenan at a 10% level in the diet. The effects of these supplements were similar to, although slightly less marked than, that of a comparable amount of pectin N.F. Cholesterol feeding also induced a slight increment in plasma cholesterol levels which was partially counteracted by concurrent feeding of pectin N.F. and several of the other test supplements. Differences between the various groups however were not statis-

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† Vitamin-Free Test Casein, General Biochemicals, Inc., Chagrin Falls, Ohio.

‡ Hubbell, Mendel and Wakeman Salt Mixture, General Biochemicals, Inc., Chagrin Falls, Ohio.

TABLE I. Effects of Dietary Supplements on Plasma and Liver Cholesterol and Liver Total Lipid of Cholesterol-Fed Rats (8 Animals/Group).*

Supplements fed with basal ration	Body wt at sacrifice, g†	Plasma cholesterol, mg/100 ml† Free	Total	Liver cholesterol, mg/g† Free	Total	Liver total lipid, %†
None	197.4 ± 4.9	20.1 ± 1.6	85.2 ± 5.8	1.63 ±.09	2.09 ± .09	4.74 ±.3
1% cholesterol	201.3 ± 6.8	21.0 ± 1.1	105.5 ± 5.0	2.72 ±.14	16.17 ±1.42	9.03 ±.7
Idem + following supplements:						
5% pectin N.F.	185.4 ± 6.1	19.6 ± 1.0	92.8 ± 4.2	2.45 ±.13	5.59 ± .64	6.31 ±.4
10% " "	181.7 ± 5.6	18.2 ± 1.4	95.6 ± 3.3	2.38 ±.17	4.12 ± .42	4.78 ±.3
10% Gum Guar	177.6 ± 5.2	20.8 ± 1.2	91.3 ± 4.3	2.29 ±.12	6.05 ± .79	6.29 ±.5
10% Locust Bean Gum	180.3 ± 5.4	24.0 ± 1.5	103.3 ± 5.0	2.38 ±.08	7.59 ± .73	6.81 ±.4
10% carrageenan	191.9 ± 3.8	20.8 ± 1.6	89.3 ± 5.5	2.22 ±.09	5.52 ± .62	5.86 ±.3

* Test supplements obtained from the following sources: pectin N.F. (citrus), Sunkist Growers, Ontario, Calif.; Gum Guar and Locust Bean Gum, Hathaway Allied Products, Los Angeles, Calif.; carrageenan (Gelcarin MR 100), Marine Collids, Inc., of America, New York. Test supplements were all natural sources of hydrophyllic complex carbohydrate colloids made up of repeating units of (1) galacturonic acid in the case of pectin, (2) mannose and galactose in Gum Guar and Locust Bean Gum, and (3) sulphated galactose in Gelcarin MR 100. The pectin N.F. employed was a purified material obtained from the dilute acid extract of the inner portion of the rind of citrus fruits. It had a methoxyl value of 10.7% on a moisture-ash-free basis. Gum Guar was the ground endosperm of Guar (known botanically as *Cyamopsis tetragonolaba*) seed with a galactomannan content of approximately 80%. Locust Bean Gum was obtained from the endosperm of the kernels of the Carob tree. It was a hemicellulose product of about 4 parts mannose and 1 part galactose. Gelcarin MR 100, a highly purified product designed for use in milk or milk products, is known chemically as carrageenan. The latter occurs naturally in a number of red seaweeds (class Rhodophyceae) but is obtained principally from the group of seaweeds known as Irish moss.

† Including stand. error of mean.

tically significant.§ Findings are summarized in Table I.

Discussion. A number of studies indicate that, in general, populations habitually subsisting on diets low in fats and animal protein tend to have a low concentration of serum cholesterol and a low incidence of cardiovascular disease(4-7). Such diets contain a number of constituents which are either absent from or present in only minute amounts in the diets of populations with a high incidence of hypercholesterolemia, atherosclerosis and coronary artery disease. Walker and Arvidsson(8) and Higginson and Pepler(9) were among the first to call attention to the high fiber content of the Bantu diet as a possible explanation for the low serum cholesterol level observed in the Bantu population. A similar suggestion was also made by Bersohn *et al.*(10). More recently Keys *et al.* (11) conducted controlled experiments in which groups of physically healthy men subsisted alternately on "American" and "Italian" types of diets, devised to be comparable in calories, proteins and in kind and amounts of fat but differing in the sources of carbohydrates. An abundance of fruits and vegetables in the Italian type diets (which tended to be high in complex carbohydrates such as pectins, hemicelluloses and fiber) replaced equivalent calories in simpler carbohydrates in the American type. Serum cholesterol lev-

§ Food intake was determined for rats in the various groups. Since the weight increment of rats fed the test supplements was less than that of rats fed the basal + cholesterol diet, the question arose whether the reduction in weight increment of rats fed the test supplements may not have been due to a reduction in amount of diet and hence cholesterol ingested and whether the reduced cholesterol intake in turn may not have been the cause of lower liver cholesterol and liver total lipid levels. Such, however, does not appear to be the case, for differences in body weight between the various groups were not statistically significant whereas differences in liver cholesterol and liver total lipid were. Furthermore, an even greater reduction in body weight was observed (unpublished findings) in rats fed the basal ration + cholesterol + 20% alfalfa meal without an accompanying reduction in liver cholesterol and liver total lipid values. The possibility that the test supplements inhibited absorption of cholesterol from the gut, however, has not been excluded.

els were significantly lower with the "Italian" type diets. In subsequent studies Keys *et al.* (12) reported that citrus pectin when fed at a level of 15 g per day caused a slight but statistically significant reduction in serum cholesterol levels in physically healthy, middle-aged men; cellulose (fiber) fed under comparable conditions was without significant effect. These studies suggest that cellulose or fiber *per se* was not responsible for the low serum cholesterol levels of the Bantu and comparable groups, but that pectin, another complex bulk-forming carbohydrate, may have had some activity in this regard.|| An analysis of the diets of native populations with low serum cholesterol levels indicated that in addition to pectin such rations also contain gums and/or other complex carbohydrates such as colloids of marine plants which were found in the present experiment to cause a highly significant reduction in liver cholesterol levels in the cholesterol-fed rat. Further studies are indicated to determine what effect these substances might have, when administered alone or in combination with one another and pectin, in treatment of hypercholesterolemia and atherosclerosis in man.

|| Unpublished studies from this laboratory indicate that different batches of pectin may vary markedly in anti-cholesterol activity. Pectic preparations with a methoxyl content of 5% or less were without activity in counteracting the increment in plasma and liver cholesterol levels induced by cholesterol feeding in the rat in contrast to the marked activity exhibited by pectin N.F. preparations of relatively high methoxyl content (10.7% on a moisture-ash-free basis).

Summary. The increment in liver cholesterol and liver total lipid induced by cholesterol feeding in the rat was largely counteracted by concurrent feeding of Gum Guar, Locust Bean Gum or carrageenan at a 10% level in the diet. Effects were similar to, although slightly less marked, than that obtained with a comparable amount of pectin N.F.

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Sequential Alterations of Lactic Dehydrogenase Isozymes During Embryonic Development and in Tissue Culture.* (27586)

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Enzymatic changes during growth and development have been extensively studied. The early chick embryo contains low phosphatase activity(1) relatively high peptidase activity(2) and very high concentrations of cytochrome oxidase and succinoxidase(3). During fetal development sequential alterations of phenylalanine hydroxylase, tyrosine transaminase and phenylalanine transaminase in rabbits(4) and of arginase in chicks(5), have been described, and each of these enzymes exhibits a characteristic pattern of

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