Comparative Effects of Pectin N. F. Administration on the Cholesterol-Fed Rabbit, Guinea Pig, Hamster and Rat.* (27730)

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Previous findings indicate that citrus and apple pectin when incorporated at a 5% level in the diet largely counteracted the increase in plasma and liver cholesterol and liver total lipids induced by cholesterol feeding in the rat(1). The activity of pectin in this regard was correlated, at least in part, with its methoxyl content since pectic substances with a methoxyl content of 5.0% or lower were devoid of activity in contrast to the marked activity exhibited by those with a methoxyl content of 10.1% or higher(2). Data are presented here on the comparative effects of pectin of relatively high methoxyl content (*i.e.*, 10.7% on a moisture-ash-free basis) on plasma and liver cholesterol and liver total lipid levels in the cholesterol-fed rabbit, guinea pig, hamster and rat.

Procedure. Male rabbits, guinea pigs, hamsters and rats were selected at the weights indicated in Table II and each species was divided into 3 comparable groups fed respectively a purified basal ration, the basal ration + 1% cholesterol, and the basal ration + 1% cholesterol + 5% pectin N.F. The composition of the diets is indicated in Table I The animals were placed in metal cages with raised screen bottoms (the rabbits in individual cages; the other species, 2 animals per cage) and were provided the test diets and water *ad libitum*. The guinea pigs were killed after 4 weeks of feeding and the other species after six. At time of autopsy the animals were anesthetized with sodium pentobarbital, and blood was withdrawn from the aorta into a heparinized syringe.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.*(3), and cholesterol was determined on liver and plasma by the method of Nieft and Deuel(4). Total lipids were determined gravimetrically on an aliquot of the liver extract.

Results. A significant difference in response was observed between the rat in contrast to the rabbit, guinea pig and hamster in respect to the effects of pectin feeding on plasma and liver cholesterol and liver total lipids following cholesterol administration. Citrus pectin with a methoxyl content of 10.7% when fed at a 5% level in the diet largely counteracted the increment in plasma and liver cholesterol and liver total lipids induced by cholesterol feeding in the rat but was without significant effect in the cholesterol-fed rabbit, guinea pig and hamster. With the exception of rabbits fed the basal + cholesterol ration, all cholesterol-fed animals gained weight during the experimental period. The increment in plasma cholesterol levels following cholesterol feeding was highest in the rabbit and least in the rat. Liver cholesterol levels after cholesterol feeding were highest in the hamster. Results are summarized in Table II.

No data are available to account for the differences in response between the various species. Since the 3 species in which pectin was without significant effect were herbivorous whereas the rat in which it was active is not, it is possible that the "anti-cholesterol" effect of pectin is confined to non-herbivorous species. Of possible pertinence in this regard is the observation of Keys *et al.*(5) that citrus pectin when fed at a level of 15 g per day caused a slight but statistically significant reduction in serum cholesterol of another non-herbivorous species, namely man. Further studies are indicated to determine to what extent differences in intestinal physiology or in the digestion or absorption of pectin between herbivorous and non-herbivirous species contributed to the diverse results.

Summary. Citrus pectin with a methoxyl content of 10.7% when fed at a 5% level in the diet largely counteracted the increment

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TABLE I. Composition of Experimental Diets.

Ingredients	Rabbit			Guinea pig			Hamster			Rat		
	A_1	A_2	A_3	B_1	B_2	B_3	C_1	C_2	C_3	D_1	D_2	D_3
Magnesium oxide				.5	.5	.5						
Potassium acetate				2.5	2.5	2.5						
Cholesterol		1	1		1	1		1	1		1	1
Salt mixture*	5	5	5	5	5	5	5	5	5	5	5	5
Casein†	25	25	25	30	30	30	24	24	24	24	24	24
Sucrose	45	44	39	37	36	31	61	60	55	61	60	55
Pectin N.F.‡			5			5			5			5
Cellulose§	10	10	10	10	10	10						
Agar				5	5	5						
Crisco	10	10	10									
Cottonseed oil	5	5	5	10	10	10	10	10	10	10	10	10

To each kg of diets A_1, A_2 and A_3, and B_1, B_2 and B_3 were added the following vitamins: thiamine hydrochloride, 16 mg; riboflavin, 16 mg; pyridoxine hydrochloride, 16 mg; calcium pantothenate, 40 mg; nicotinic acid, 200 mg; biotin, 1 mg; folic acid, 10 mg; vit. B_{12}, 100 μg; 2-methyl-1,4 naphthoquinone, 5 mg; para-aminobenzoic acid, 100 mg; ascorbic acid, 1.5 g; choline chloride, 2 g; vit. A, 5000 U.S.P. units; vit. D_2, 500 U.S.P. units; and alpha-tocopherol acetate, 100 mg. To each kg of diet A_1, A_2 and A_3 were added 200 mg of inositol; and to each kg of diets B_1, B_2 and B_3 were added 2 g of inositol. The vitamins were added in place of an equal amount of sucrose. Each guinea pig also received daily (6 times/wk) an oral supplement of 10 mg ascorbic acid in 0.1 ml water.

To each kg of diets C_1, C_2 and C_3, and D_1, D_2 and D_3 were added the following vitamins: 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; para-aminobenzoic acid, 400 mg; inositol, 800 mg; 2-methyl-1,4 naphthoquinone, 5 mg; vit. B_{12}, 150 μg; vit. A, 5000 U.S.P. units; vit. D_2, 500 U.S.P. units; and alpha-tocopherol acetate, 100 mg. The vitamins were added in place of an equal amount of sucrose.

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

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

‡ Citrus pectin N.F. (with a methoxyl content of 10.7% on a moisture-ash-free basis). This material was kindly provided by Dr. Glenn H. Joseph and Mr. Willard E. Baier of Sunkist Growers, Ontario, Calif.

§ Cellulose (Solka Floc BW 200), Brown Co., Boston, Mass.

TABLE II. Comparative Effects of Pectin Feeding on Plasma and Liver Cholesterol and Liver Total Lipid Levels in Cholesterol-Fed Rabbit, Guinea Pig, Hamster and Rat.*†

Group	Body wt, g		Plasma cholesterol, mg/100 ml‡		Liver wt, g‡	Liver total lipid, %‡	Liver cholesterol, mg/g‡	
	Initial	Final	Free	Total			Free	Total
Rabbit								
Diet A_1	2140	2463	42.3 ± 8.3	159 ± 30	72.0 ± 3.6	4.2 ± .3	2.18 ±.09	3.4 ± .4
" A_2	2179	1655	610 ± 77	1363 ±244	54.6 ± 8.4	7.0 ±1.0	5.77 ±.52	21.1 ± 6.2
" A_3	2172	2408	512 ± 75	1563 ±192	79.3 ± 4.1	11.0 ± .8	5.08 ±.52	31.8 ± 5.2
Guinea pig								
Diet B_1	295	494	10.6 ± 1.0	53 ± 4	21.9 ± 1.4	4.1 ± .1	2.09 ±.06	2.3 ± .1
" B_2	296	399	149 ± 11	398 ± 24	28.3 ± 1.1	13.9 ± .7	6.59 ±.35	27.6 ± 2.6
" B_3	294	387	129 ± 11	326 ± 27	28.8 ± 1.2	14.4 ± .9	6.55 ±.40	25.9 ± 3.3
Hamster								
Diet C_1	39	97	39.6 ± 2.6	132 ± 7	5.5 ± .2	3.9 ± .1	2.08 ±.07	2.3 ± .1
" C_2	39	89	140 ± 10	495 ± 32	7.3 ± .2	13.9 ± .3	6.36 ±.30	66.3 ± 1.8
" C_3	39	96	130 ± 10	435 ± 39	7.4 ± .3	14.6 ± .5	6.36 ±.20	71.6 ± 3.3
Rat								
Diet D_1	44	284	19.8 ± .9	95 ± 4	10.6 ± .2	4.5 ± .1	1.83 ±.03	2.3 ± .1
" D_2	45	294	26.6 ± 2.2	145 ± 8	13.3 ± .5	12.6 ± .9	4.06 ±.25	31.1 ± 3.4
" D_3	45	293	20.0 ± 1.6	104 ± 7	12.5 ± .5	7.3 ± .4	2.84 ±.16	10.8 ± 1.8

* Each dietary group of rabbits consisted of 6 animals; each dietary group of guinea pigs, hamsters and rats (Holtzman strain) consisted of 10 animals.

† Guinea pigs were sacrificed after 4 wk of feeding; rabbits, hamsters and rats after 6 wk.

‡ Including stand. error of mean.

in plasma and liver cholesterol and liver total lipids induced by cholesterol feeding in the rat but was without significant effect in the cholesterol-fed rabbit, guinea pig and hamster.

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Carbohydrate Metabolism in Strain L-929 Mouse Fibroblast Cells.*† (27731)

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The L-strain of mouse fibroblast has been used extensively in studies of the mechanism of viral infection. However, little information is available concerning the metabolism of this cell line or the effects of viral infection on its metabolism. It is particularly well suited to biochemical investigation because of its ability to grow rapidly in suspension culture in relatively simple media.

Current work on the biochemistry of cultured mammalian cells has been reviewed by Levintow and Eagle(1). Phillips and Andrews (2) have reported experiments on the effect of various factors on respiratory activity in L-cells. The production of lactic acid by this cell line has been correlated with the growth phase of the cells by Munyon and Merchant (3).

The present study represents a survey of the enzymic constitution of L-cells, with respect to enzymes involved in the intermediary metabolism of carbohydrate, undertaken as a preliminary to an investigation of the effects of vaccinia-virus infection on the carbohydrate metabolism of this cell line.

Methods. The strain L-929 mouse fibroblast cell line was cultured in suspension in YELP medium(4), with 0.12% Methocel, 100 μg/ml streptomycin, and 100 units/ml penicillin. The medium was contained in screw-cap Erlenmeyer flasks and agitated on a rotary shaker at 37°C. New cultures were started by dilution of late log phase cultures (having a cell concentration of between 1.0 and 1.3 $\times$ 10^6 cells per ml) with sufficient fresh medium to reduce the cell count to about 2.5 $\times$ 10^5 cells per ml. When the desired cell concentration had been attained, cultures were harvested by centrifugation at room temperature. Cells used for measurements of respiratory activity (harvested at a cell count of 6-8 $\times$ 10^5 cells per ml) were washed twice with Krebs-Ringer-phosphate solution and then suspended in this solution at a concentration of 5 $\times$ 10^6 cells per ml. Cell extracts used in Warburg experiments were prepared by sonication (as described below) of a suspension containing 2 $\times$ 10^7 cells per ml of Krebs-Ringer-phosphate solution. Conventional manometric technics were employed for measurement of oxygen uptake at 37°C with the Warburg constant volume respirometer. All materials added to Warburg flasks were dissolved in Krebs-Ringer-phosphate solution, and the pH of the solutions adjusted to pH 7.2-7.4.

Cells used for measurements of enzymic activity (harvested at a cell concentration of approximately 1 $\times$ 10^6 cells per ml) were washed twice in Krebs-Ringer-phosphate so-

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