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Effect of N-(1-Methyl-2,3,di-p-chlorophenylpropyl)-Maleamic Acid (Benzmalecene) on Serum Lipids and Lipoproteins.* (25497)

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Among hypocholesterolemic agents recently made available for clinical trial is N-(1-methyl - 2,3,di-p-chlorophenylpropyl) - maleamic acid, Benzmalecene,† said to inhibit incorporation of 1-C^{14} acetate and 2-C^{14} mevalonic acid into cholesterol by rat liver homogenates, to inhibit development of hypercholesterolemia in chickens fed a diet containing 2% cholesterol and 5% cottonseed oil, to reduce the magnitude of cholesterolemia in rats fed a natural diet to which saturated fat was added, and to reduce serum cholesterol levels in dogs maintained on standard laboratory rations(1). We administered Benzmalecene to one hypercholesterolemic human subject and 2 normocholesterolemic subjects and noted hypocholesterolemic effects in each. Unusual changes in serum lipid pattern were induced.

Methods. Serum total and unesterified cholesterol and phospholipid concentrations and cholesterol and phospholipid content of serum lipoprotein fractions obtained by differential preparative ultracentrifugation were determined by methods we previously reported(2). "Phospholipids" were calculated by multiplying lipid P values by 25. Serum triglycerides were determined by the method of Van Handel and Zilversmit(3). Benzmalecene was administered to the following subjects: (1) PB a 39 year old woman with familial hypercholesterolemia who received 1 g/day for 7 days followed by 1.5 g/day for next 7 days; (2) ABB a 41 year old man with Klinefelter's syndrome who received 1.5 g/day for 10 days; (3) GM a 27 year old man with myotonic dystrophy and hypogonadism who received 1.5 g/day for 12 days. Both pre- and post-treatment control sera were studied. Post-treatment sera were obtained beginning 12 to 21 days after cessation of treatment.

Results. No change in serum transaminase (glutamic-oxaloacetic and glutamic-pyruvic) or van den Bergh levels were noted during the relatively short period of study. (Bromsulphalein retention has been noted in chronic toxicity studies in animals(1)). The compound uniformly produced anorexia or nausea which prompted one subject to refuse further medication after 12 days of treatment. No dietary restrictions were imposed and no changes in weight were noted. Since weight loss did not occur, the changes in serum lipids are not attributable to reduced caloric intake. The influence of anorexia *per se* on serum lipids is unknown.

The effects on serum lipids were similar in all subjects (Table I). The decrease in serum total cholesterol was limited to esterified cholesterol. Analysis of the ultracentrifugally separated lipoprotein fractions of densities <1.063 g/ml (includes "β" and all lower

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† Merck, Sharp and Dohme Research Labs., West Point, Pa.

TABLE I. Serum Lipid Values, mg %.

Subject	No. of samples	Rx	Cholesterol Total	Cholesterol Free	Cholesterol Ester	% ester	Phospho-lipids	C/P	Tri-glycerides
PB	5	Control	446	127	319	72	397	1.12	111.5
	3	1 g/day	411	126	285	69	401	1.00	199.8
	4	1.5 "	351	121	229	65	421	.83	337.4
	8	Post Rx	418	120	298	71	368	1.14	106.5
ABB	4	Control	281	71	211	75	301	.93	108.9
	3	1.5 g/day	212	102	110	52	385	.55	513.0
	3	Post Rx	263	67	196	74	276	.95	96.6
GM	2	Control	257	67	189	75	288	.89	103.7
	3	1.5 g/day	204	71	133	66	293	.70	244.7
	4	Post Rx	243	65	178	73	291	.83	113.7

density lipoproteins) and >1.063 g/ml ("α" lipoproteins) (Table II) revealed that the esterified cholesterol content of both fractions was decreased.

Of great interest is the increase in serum phospholipid and triglyceride levels. Increase in serum phospholipids and decrease in serum cholesterol levels resulted in marked decrease in value for C/P ratio. Lipoprotein analysis revealed that the increase in serum phospholipids was due to increase in lipid P content of the lower density (<1.063 g/ml) lipoprotein fraction.

In subject PB, lipoproteins of density < 1.063 g/ml were further subdivided into fractions of density >1.019<1.063 g/ml (β lipoproteins) and density <1.019 ("low density β lipoproteins" plus very low density lipoproteins). Values are shown in Table III.

Analysis of β lipoprotein fraction revealed a diminished content of cholesterol and lipid P. In the lipoprotein fraction of density< 1.019 g/ml, sizeable increments in both cholesterol and lipid P content were noted. The portion of serum cholesterol and lipid P in this low density fraction had more than dou-

TABLE II. Cholesterol and Phospholipid Content (mg %) of Lipoprotein Fractions of Densities >1.063 (α) and <1.063 mg/ml.*†

Subject	Rx	Total cholesterol >1.063	Total cholesterol <1.063	% esterified >1.063	% esterified <1.063	Phospholipid >1.063	Phospholipid <1.063	C/P ratio >1.063	C/P ratio <1.063
PB	Control	54	391	79	71	96	268	.50	1.34
	1 g/day	36	399	80	72	88	284	.41	1.41
	1.5 "	21	317	74	65	87	307	.24	1.03
	Post Rx	50	396	81	73	99	256	.51	1.55
ABB	Control	34	255	91	76	99	177	.35	1.45
	1.5 g/day	14	242	77	54	84	277	.17	.75
	Post Rx	48	234	81	75	106	158	.45	1.48
GM	Control	61	208	84	74	133	142	.46	1.45
	1.5 g/day	24	190	81	69	91	178	.26	1.07
	Post Rx	50	202	84	74	126	160	.39	1.27

* Cholesterol and phospholipid content of lipoproteins reported as mg lipid in designated lipoprotein fraction present in 100 ml serum.

† No. of samples of sera subjected to ultracentrifugal fractionation is the same as or slightly less than No. of samples listed for each subject in Table I.

TABLE III. Cholesterol and Phospholipid Content (mg %) of Lipoprotein Fractions of Densities >1.019 <1.063 and <1.019 (β) g/ml. Subject PB.

Rx	Total cholesterol >1.019 <1.063	Total cholesterol <1.019	% esterified >1.019 <1.063	% esterified <1.019	Phospholipid >1.019 <1.063	Phospholipid <1.019
Control	305	55	71	69	218	51
1 g/day	278	122	74	70	196	88
1.5 "	173	144	64	66	172	135
Post Rx	343	52	73	73	219	36

bled by the end of 14th day of treatment.

Summary. Administration of Benzmalecene alters serum lipids as follows: 1) decreases serum total and esterified cholesterol concentrations; 2) decreases cholesterol and phospholipid content of α (density >1.063 g/ml) and β (density $>1.019<1.063$ g/ml) lipoproteins; 3) increases serum phospholipid and triglyceride concentrations; 4) increases, both absolutely and relatively, cholesterol and phospholipid content of lipoproteins of density <1.019 g/ml; and, 5) decreases C/P ratio in whole serum and in α and β lipoproteins. The compound possesses extraordinary lipid-altering properties which warrant further investigation. However, the increase in low density lipoprotein lipids may be an undesirable effect since low density lipoproteins may play a role in atherogenesis.

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Effect of Phytic Acid on Zinc Availability.* (25498)

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Considering low zinc requirement of laboratory animals fed casein as source of protein it is surprising that, in recent years, deficiency symptoms have appeared in animals maintained under practical conditions(1). This has probably come about because of more common use of soybean meal in rations inasmuch as several investigators have found zinc in soy protein less available than that in animal proteins(2,3,4). Evidence has been presented that heat treatment increases availability of zinc in soy protein(5,6) although this observation has not been confirmed under our laboratory conditions. Phytin accounts for about 70% of phosphorus in soybean meal, and during extraction of soy protein, phytic acid forms a complex with protein(7). That isolated soy protein has a high affinity for zinc led us to test whether or not a casein-phytic acid complex would decrease availability of zinc to the growing chick.

Methods and material. To prepare casein-phytic acid complex, casein was suspended in distilled water to make a slurry, and phytic acid was added equivalent to 5% of dry weight. After standing overnight the mixture was dried in oven at 60°C. A similar complex was prepared with soy protein† using 4% of phytic acid. Phytic acid analyses were performed by the method of Pringle and Moran(8) except that samples were extracted twice for 18 hours. The original soy protein contained 0.5% of phytic acid phosphorus and the casein-phytic acid complex, 0.8%. According to analysis about one-fourth of phosphorus in commercial phytic acid was inorganic, and total phosphorus contents of proteins were: soy protein 0.9%; casein 0.7%; casein-phytic complex 1.8%. Basal diet, type of chicks and experimental conditions were as previously described(9) except that the battery was plastic coated. Other diets fed were modifications of the soy protein basal. Twenty-six % of soy protein-phytic acid complex, 18% of casein and 5% of gelatin, or 19% of casein-phytic acid complex and 5% of gelatin were substituted for soy protein in the respective diets and glucose hydrate was adjusted accordingly. Other additions were made at the expense of glucose.

Results of 2 trials are summarized in Table I. Rate of growth on casein-gelatin basal diet

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†C-1 Assay Protein, Archer-Daniels-Midland Co., Cincinnati, O.