EFFECT OF SOME WHEAT MILL-FRACTIONS ON BLOOD AND LIVER LIPIDS IN CHOLESTEROL-FED RATS[1,2]

G. S. RANHOTRA, R. J. LOEWE, and L. V. PUYAT, Nutrition Laboratory, American Institute of Baking, 400 E. Ontario, Chicago, IL 60611

ABSTRACT

Young male rats fed an atherogenic diet containing cholesterol showed, at 2 weeks, a sharp increase in their serum cholesterol levels which gradually declined over the next 6 weeks. When sucrose in the diet (50%) was substituted, in entirety, with three wheat fractions (flour, germ, and bran), elevation in serum cholesterol levels was significantly less pronounced throughout, in germ- and bran-fed rats, and, to a lesser extent, in those fed patent flour. This occurred in bran-fed rats in spite of higher intake of cholesterol. Effect on serum triglyceride levels was inconsistent; still, some lowering of levels was observed in flour- and germ-fed rats. In rats fed a cholesterol-free diet, substitution of sucrose with wheat fractions did not lower serum cholesterol levels, and some reduction in triglyceride levels occurred only in flour-fed rats. Livers of cholesterol-fed rats showed marked infiltration of cholesterol and increase in weights; these changes, however, were much less pronounced in germ- and bran-fed rats. Liver triglycerides were little affected. In cholesterol-fed rats, substituting sucrose with bran lowered chylomicron and β-lipoprotein fractions, and some reduction in β- and pre-β-fractions was also observed in rats fed patent and whole wheat flours.

Cereal Chemistry 53(4): 540-548

Several risk factors involved in the etiology of atherosclerosis have been identified and well documented. Based on epidemiological studies in human populations as well as experiments with animals, elevated blood lipid levels, especially cholesterol levels, have been shown to be one of the factors strongly associated with increased susceptibility to atherosclerosis (1–6). The use of lipid-lowering diets can considerably delay an early onset of atherosclerosis (4–7). Cereals, leguminous seeds, fruits, and vegetables form important ingredients of a lipid-lowering diet and the staples of population groups showing characteristic low plasma lipid levels (1–4); what fraction, chemically defined, of this diet is mainly responsible for the observed effect has not been conclusively shown. Polyunsaturates, protein, fiber, minerals, etc. have been implicated (3,4,8–12). Present work was undertaken to examine three main fractions of a staple cereal,

[1]Presented at the 60th Annual Meeting, Kansas City, Mo., Oct. 1975.

[2]This study was supported by the U.S. Department of Agriculture in cooperation with the National Wheat Institute, Contract No. NWI-01, administered by the National Wheat Institute, Washington, D.C.

wheat, for their possible lipid-lowering effect; follow-up studies would attempt to identify subfractions and would also examine the possible mode of action of those showing hypolipidemic effect.

MATERIALS AND METHODS

Tempered wheat (hard red winter) and resultant patent flour, germ, and bran

TABLE I
Proximate Composition

	Wheat			
	Flour			
	Patent	Whole	Germ	Bran
Moisture, %	12.4	7.9	7.4	8.8
Protein[a], %	10.9	12.9	26.3	15.5
Ash, %	0.5	2.0	4.8	6.6
Ether extract, %	0.9	1.6	9.1	4.0
Carbohydrates[b], %	75.3	75.6	52.4	65.1

[a]Conversion (N ×) factors used; patent flour, 5.70; whole wheat flour, 5.83; germ, 5.80; and bran, 6.31.
[b]By difference (subfractions not determined).

TABLE II
Composition of Experimental Diets

			Wheat							
			Flour							
	Sucrose		Patent		Whole		Germ		Bran	
	A[a]	B	C	D	E	F	G	H	I	J
Ingredients, %										
Casein[b]	25	25	25	25	25	25	25	25	25	25
Lard	16.7	16.7	16.7	16.7	16.7	16.7	16.7	16.7	16.7	16.7
NaCl	1	1	1	1	1	1	1	1	1	1
Vitamins[c]	2	2	2	2	2	2	2	2	2	2
Minerals[d]	4	4	4	4	4	4	4	4	4	4
Starch	1.3	...	1.3	...	1.3	...	1.3	...	1.3	...
Cholesterol	...	1	...	1	...	1	...	1	...	1
Cholic acid	...	0.3	...	0.3	...	0.3	...	0.3	...	0.3
Sucrose	50	50	...	...	...	...	...	...	...	...
Wheat/fraction	...	...	50	50	50	50	50	50	50	50
Composition, %										
Protein[e]	21.3	21.1	26.9	26.7	28.1	27.9	35.1	35.0	29.6	29.4
Ether extract	16.9	18.2	17.3	18.3	17.7	19.1	21.7	22.8	19.0	20.7

[a]The letters A–J indicate the diets used.
[b]From ICN Pharmaceuticals, Cleveland, Ohio.
[c]Vitamin diet fortification mixture from ICN Pharmaceuticals.
[d]Salt 446 from ICN Pharmaceuticals (H. Spector. J. Biol. Chem. 173: 659 (1948)).
[e]Conversion (N×) factors used: casein, 6.25; others, as in Table I.

TABLE III
Effect of Wheat and its Fractions on Lipid Levels[a]

				Wheat							
				Flour							
		Sucrose		Patent		Whole		Germ		Bran	
	Weeks	A[b]	B	C	D	E	F	G	H	I	J
Serum cholesterol[c], mg/100 ml	2	108 ±10	505 ±28	144 ±6	674 ±55	164 ±19	756 ±77	158 ±11	305 ±40	191 ±8	409 ±53
	4	100 ±11	400 ±25	110 ±12	434 ±35	141 ±12	666 ±99	129 ±8	235 ±25	178 ±21	333 ±45
	6	105 ±16	368 ±72	102 ±10	257 ±19	109 ±14	368 ±98	106 ±9	159 ±32	156 ±16	257 ±25
	8	130 ±13	297 ±48	111 ±14	227 ±37	131 ±20	303 ±42	119 ±8	186 ±24	167 ±21	224 ±36
Serum TG[c], mg/100 ml	2	115 ±9	108 ±25	97 ±15	101 ±14	128 ±29	96 ±24	82 ±12	68 ±11	120 ±17	122 ±14
	4	173 ±46	146 ±56	141 ±26	123 ±25	186 ±46	143 ±42	163 ±33	114 ±29	148 ±25	121 ±32
	6	116 ±42	110 ±40	125 ±61	92 ±26	146 ±34	147 ±44	157 ±112	53 ±34	160 ±60	112 ±39
	8	205 ±41	112 ±33	64 ±35	146 ±35	283 ±93	169 ±45	248 ±51	159 ±58	169 ±27	136 ±23
Liver cholesterol[c], mg/g liver	8	2.0 ± 0.5	67.6 ± 5.3	2.2 ± 0.6	66.1 ± 8.8	2.2 ± 0.6	63.6 ± 4.7	2.3 ±0.5	39.7 ± 7.9	2.3 ± 0.4	55.7 ± 6.9
Liver TG[c], mg/g liver	8	1.6 ± 0.1	2.3 ± 0.5	1.8 ± 0.3	2.3 ± 0.3	1.7 ± 0.3	2.3 ± 0.3	1.8 ±0.2	2.7 ± 0.6	1.7 ± 0.2	2.3 ± 0.5
Liver protein, g/100 g liver	8	17.5	14.1	18.1	13.2	18.6	12.0	18.2	13.1	18.1	12.0

[a]Values represent the average of 8 rats ± standard deviation.
[b]The letters A–J indicate the diets used.
[c]Initial (0-day) values: serum cholesterol, 122 ± 12; serum TG, 39 ± 2; liver cholesterol, 2.2 ± 0.1; liver TG, 2.6 ± 0.4.

were obtained from a commercial mill (Dixie-Portland), air-dried, and ground, excepting flour, to fine particle size (0.024-in. screen) before incorporation into test diets. Table I lists proximate composition of wheat and its fractions used. Diets were formulated with and without added cholesterol and cholic acid, and compared against the atherogenic (sucrose-based) diet (Table II).

Male, weanling, Sprague-Dawley rats (eight per diet) weighing about 45 g initially, and individually housed, were fed diets for 8 weeks. Diet and water were offered *ad libitum*. At 2-week intervals, all rats were anesthetized and blood was withdrawn by heart puncture. Resultant serum was then used for analyses. At the end of 8 weeks, all rats were sacrificed after blood had been withdrawn. Livers were removed, thoroughly washed, blotted dry, weighed, homogenized in a Potter-Elvehjem homogenizer, and their volume recorded.

Total cholesterol in serum, liver, and diets was determined by the method of Abell *et al.* (13). Triglycerides (TG) in serum and in liver were determined by the method of Van Handel *et al.* (14) as modified by Van Handel and Ordway (15). Serum lipoproteins were separated by polyacrylamide gel electrophoresis by the method of Frings *et al.* (16). Standard AOAC methods (17) were used to determine moisture, ash, protein, and fat contents.

RESULTS AND DISCUSSION

In the commercial milling of wheat, a number of fractions are produced (18). However, only three fractions (Table I) representing the main morphological regions of the wheat kernel were compared against the sucrose-based diet (Table II).

Feeding the sucrose-based diet (diet B) caused a sharp increase, at 2 weeks, in the serum cholesterol levels (Table III). A diet high in sucrose (19), casein (9), and animal fat (8), and containing cholesterol and cholic acid (20), is reported to induce a rapid hypercholesteremia. Over the next 6 weeks, elevated serum cholesterol levels gradually declined. When sucrose was substituted in entirety (Table II) with germ, cholesterol levels were significantly ($P < 0.01$) less elevated (diet B *vs.* H) all through the test period (Table III). This may be due to high levels of polyunsaturates (Tables I and II) which are reported to increase the excretion of neutral steroids and bile acids (7,21,22). It remains to be shown whether defatted germ is also involved in this effect. Though less marked, a somewhat similar response was obtained with bran (diet B *vs.* J). This occurred in spite of significantly ($P < 0.05$) higher intake of bran and consequently of cholesterol (Tables II–IV). High bran intake did not increase the growth rate of rats, probably because casein alone adequately met the protein need, or because bran interfered with the availability of protein (23) and other nutrients. Final body weights of rats on all diets differed little; this eliminated, as was intended, the body weight as a factor influencing serum lipid levels.

Not enough is known about the composition and chemistry of the fiber complex in bran to attribute to it any single mode of action (10,24–26). The effect may be caused by increased excretion of fecal steroids and bile acids (4,27–30), decreased synthesis of endogenous cholesterol, or some other mechanism. Absence of effect of fiber has also been reported (31–33)—high-fiber cereal fed to healthy young adults showing normal lipid levels had no lipid-lowering effect (33). As results in Table III show (diet I), and as has also been pointed out by

TABLE IV
Diet Intake and Body and Liver Weights[a]

				Wheat							
				Flour							
		Sucrose		Patent		Whole		Germ		Bran	
	Weeks	A[b]	B	C	D	E	F	G	H	I	J
Diet intake, g	2	109 ±9	116 ±10	120 ±7	122 ±9	124 ±12	129 ±13	128 ±14	121 ±11	129 ±14	136 ±11
	4	164 ±14	181 ±27	178 ±6	166 ±16	184 ±14	196 ±17	175 ±13	180 ±12	193 ±14	206 ±18
	6	180 ±14	184 ±15	196 ±8	191 ±12	200 ±10	204 ±20	185 ±9	196 ±12	221 ±16	240 ±18
	8	183 ±14	196 ±7	211 ±13	202 ±18	214 ±17	218 ±19	195 ±14	209 ±17	243 ±15	245 ±19
Body weight, g	2	113 ±7	114 ±10	119 ±7	121 ±7	117 ±8	118 ±5	120 ±6	116 ±6	109 ±5	113 ±6
	4	198 ±14	207 ±16	209 ±11	209 ±17	209 ±14	211 ±9	209 ±12	206 ±3	190 ±10	203 ±13
	6	266 ±19	273 ±15	283 ±12	281 ±21	286 ±12	287 ±12	280 ±16	284 ±13	262 ±10	270 ±5
	8	301 ±29	324 ±18	330 ±18	327 ±28	330 ±12	336 ±11	330 ±20	337 ±22	308 ±9	323 ±19
Liver weight, g	8	12.8 ± 0.8	21.9 ± 1.1	12.7 ± 0.7	20.7 ± 2.7	12.9 ± 1.2	22.0 ± 1.4	12.7 ± 1.0	16.8 ± 1.3	13.0 ± 0.8	18.5 ± 1.4

[a]Values represent the average of 8 rats ± standard deviation.
[b]The letters A–J indicate the diets used.

TABLE V
Serum Lipoproteins (End of Eighth Week)[a]

	Diet No.									
	A	B	C	D	E	F	G	H	I	J
Top cream layer[b]	yes	no	yes	no	yes	no	yes	no	no	no
Chylomicrons	++	++++	++	++++	++	++++	++	++++	+	+
Pre-β(s)	+++	++++	++	+++	++	+++	+++	++++	+++	++++
β(s)	++++	++	+++	+	+++	+	+++	++	+++	+
α(s)	++++	++	++++	++	++++	++	++++	++	++++	++

[a]Negligible, +; faint, ++; strong, +++; very strong, ++++.
[b]Serum on standing in refrigerator; all samples were cloudy-milky.

others (34,35), this may be because it is difficult to lower a level which is already normal; bran showed a definite serum cholesterol-lowering effect when levels were sufficiently elevated (diet B *vs.* J). It has been suggested (36) that in assessing fiber, the role of its pectic and hemicellulose fractions should also be considered (37,38), since purified cellulose and lignin, the main constituents of crude fiber, contrary to the report of some (39–41), are reported to be ineffective as cholesterol-lowering agents (42–44).

Unlike germ and bran, patent flour showed a cholesterol-lowering effect after the fourth week only (diet B *vs.* D), when levels were comparable to those obtained with bran; whether starch and gluten are both effective, and over an extended period, needs investigation (22). In short-term studies with young men, an excess consumption of energy was associated with hyperlipidemia when sucrose replaced starch in the diet (19). Others (45–47) have reported similar results with starch and wheat bread fed to humans.

In agreement with the results of others (31), whole wheat flour did not lower serum cholesterol levels (diet B *vs.* F); wheat protein concentrate (fiber, about 3%) showed a similar absence of effect earlier (43). It could be that whole wheat probably shows a late effect, as results in Table III seem to suggest, and as has been reported for Bengal gram (*Cicer arietinum*) fed to humans (48). Edwards *et al.* (5), however, showed some lipid-lowering effects in normal young male subjects fed wheat.

In rats fed cholesterol-free diets, replacement of sucrose (diet A) with wheat and its fractions (diets C, E, G, and I) did not lower serum cholesterol levels; in fact, levels were somewhat elevated, especially in bran-fed rats, as others (33) have also reported with humans.

In cholesterol-fed rats, marked infiltration of liver with cholesterol and a reduction in its protein content were noted (Table III). Besides cholesterol accumulation, reduction in cytoplasmic mass may have been due to impaired protein synthesis (49). Liver TG levels changed little, probably because fatty-acid synthesis in cholesterol-fed rats is also depressed (49). Cholesterol accumulation alone fails to account for the significant ($P < 0.01$) increase observed in liver weights (Table IV); apparently cholesterol feeding also caused an increased retention of water. Replacement of sucrose with germ and bran significantly ($P < 0.01$) lowered liver cholesterol. Some effect due to whole wheat was also observed, and earlier, such an effect due to wheat protein concentrate was noted (43). No cholesterol accumulation occurred in rats fed cholesterol-free diets (Table III).

Unlike cholesterol, serum TG (which is now implicated as an additional risk factor in atherosclerosis (50)), was much less affected, for great variations between and within diets and from period to period were noted (Table III). This may be because TG and cholesterol levels usually respond in the opposite direction to low- and high-carbohydrate diets; high-carbohydrate diet usually lowers cholesterol as it raises TG (50). Some lowering of TG observed with patent flour was probably due to slower, more sustained (in comparison to sucrose) rise of blood glucose, and consequently less availability of substrate for TG synthesis (51). Germ also showed some TG-lowering effect, but liver level which slightly increased due to cholesterol-feeding remained unaffected due to diets.

The α-fraction, which is the main serum lipoprotein in rats (52), and the β-fraction appeared to be stronger in uninduced rats, suggesting a preponderance of phospholipid- and cholesterol-rich fractions (Table V). Substituting sucrose with wheat and its fractions did not affect the α-fraction; the β was reduced somewhat in uninduced rats and, except in germ-fed rats, in induced rats as well. Some reduction in the pre-β-fraction also occurred in flour-fed rats, while chylomicrons decreased in bran-fed rats only.

Although these results suggest that nonlipid dietary constituents like complex carbohydrates, both cellulosic and noncellulosic, need detailed investigation, great caution must be exercised in extending results of animal studies to man.

Literature Cited

1. HARDINGE, M. G., and STARE, F. J. Nutritional studies of vegetarians. II. Dietary and serum levels of cholesterol. Amer. J. Clin. Nutr. 2: 83 (1954).
2. HARDINGE, M. G., CHAMBERS, A. C., CROOKS, H., and STARE, F. J. Nutritional studies of vegetarians. III. Dietary levels of fiber. Amer. J. Clin. Nutr. 6: 523 (1958).
3. KEYS, A., GRANDE, F., and ANDERSON, J. F. Fiber and pectin in the diet and serum cholesterol concentration in man. Proc. Soc. Exp. Biol. Med. 106: 555 (1961).
4. TROWELL, H. Ischemic heart disease and dietary fiber. Amer. J. Clin. Nutr. 25: 926 (1972).
5. EDWARDS, C. H., BOOKER, L. K., RUMPH, C. H., WRIGHT, W. G., and GANAPATHY, S. N. Utilization of wheat by adult man: Nitrogen metabolism, plasma amino acids and lipids. Amer. J. Clin. Nutr. 24: 181 (1971).
6. MIETTINEN, M., TURPEINEN, O., KARVONEN, M., ELOSUO, R., and PAAVILAINEN, E. Effect of cholesterol-lowering diet in mortality from coronary heart-disease and other causes. Lancet 2: 835 (1972).
7. MATTSON, F. H., ERICKSON, B. A., and KLINGMAN, A. M. Effect of dietary cholesterol on serum cholesterol in man. Amer. J. Clin. Nutr. 25: 589 (1972).
8. KUMMEROW, F. A. Symposium: Nutritional perspectives and atherosclerosis. Lipids in atherosclerosis. J. Food Sci. 40: 12 (1975).
9. CARROLL, K. K., and HAMILTON, R. M. G. Symposium: Nutritional perspectives and atherosclerosis. Effects of dietary protein and carbohydrate on plasma cholesterol levels in relation to atherosclerosis. J. Food Sci. 40: 18 (1975).
10. KRITCHEVSKY, D., TEPPER, S. A., and STORY, J. A. Symposium: Nutritional perspectives and atherosclerosis. Nonnutritive fiber and lipid metabolism. J. Food Sci. 40: 8 (1975).
11. PERRY, H. M., Jr. Minerals in cardiovascular disease. J. Amer. Diet. Ass. 62: 631 (1973).
12. KLEVAY, L. M. Hypercholesterolemia in rats produced by an increase in the ratio of zinc to copper ingested. Amer. J. Clin. Nutr. 26: 1060 (1973).
13. ABELL, L., LEVY, B. B., BRODIE, B. B., and KENDALL, F. E. A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. J. Biol. Chem. 195: 357 (1952).
14. VAN HANDEL, E., ZILVERSMIT, D. B., and BOWMAN, R. Micromethod for the direct determination of serum triglycerides. J. Lab. Clin. Med. 50: 152 (1957).
15. VAN HANDEL, E., and ORDWAY, R. Suggested modifications of the microdetermination of triglycerides. Clin. Chem. 7: 249 (1961).
16. FRINGS, C. S., FOSTER, L. B., and COHEN, P. S. Electrophoretic separation of serum lipoproteins in polyacrylamide gel. Clin. Chem. 17: 111 (1971).
17. ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. Official methods of analysis (12th ed.). The Association: Washington, D.C. (1975).
18. PYLER, E. J. Baking science and technology (Vol. I). Siebel Pub. Co.: Chicago (1973).
19. ANONYMOUS. Sucrose and hyperlipidemia. Nutr. Rev. 33: 44 (1975).
20. KIRIYAMA, S., OKAZAKI, Y., and YOSHIDA, A. Hypocholesterolemic effect of polysaccharides and polysaccharide-rich foodstuffs in cholesterol-fed rats. J. Nutr. 97: 382 (1969).
21. BROWN, H. B. Food patterns that lower blood lipids in man. J. Amer. Diet. Ass. 58: 303 (1971).
22. GROEN, J. J. Why bread in the diet lowers serum cholesterol. Proc. Nutr. Soc. 32: 159 (1973).
23. RANHOTRA, G. S., HEPBURN, F. N., and BRADLEY, W. B. Effect of fiber on the availability of protein from wheat shorts. Cereal Chem. 48: 9 (1971).
24. SPILLER, G. A., and AMEN, R. J. Research on dietary fiber. Lancet 2: 1259 (1974).

25. EASTWOOD, M. A. Dietary fibre in human nutrition. J. Sci. Food Agr. 25: 1523 (1974).
26. SPILLER, G. A., and AMEN, R. J. Plant fibers in nutrition: Need for better nomenclature. Amer. J. Clin. Nutr. 28: 675 (1975).
27. BALMER, J., and ZILVERSMIT, D. B. Effects of dietary roughage on cholesterol absorption, cholesterol turnover and steroid excretion in the rat. J. Nutr. 104: 1319 (1974).
28. KRITCHEVSKY, D., and STORY, J. A. Binding of bile salts in vitro by nonnutritive fiber. J. Nutr. 104: 458 (1974).
29. MORGAN, B., HEALD, M., ATKIN, S. D., and GREEN, J. Dietary fibre and sterol metabolism in the rat. Brit. J. Nutr. 32: 447 (1974).
30. BROWN, H. B., and FARRAND, M. What a dietitian should know about hyperlipidemia. J. Amer. Diet. Ass. 63: 169 (1973).
31. TRUSWELL, A. S., and KAY, R. M. Absence of effect of bran on blood-lipids. Lancet 1: 922 (1975).
32. EASTWOOD, M. Dietary fibre and serum lipids. Lancet 2: 1222 (1969).
33. CONNELL, A. M., SMITH, C. L., and SOMSEL, M. Absence of effect of bran on blood-lipids. Lancet 1: 496 (1975).
34. HEATON, K. W., and POMARE, E. W. Bran and blood-lipids. Lancet 1: 800 (1975).
35. TROWELL, H. Bran and blood-lipids. Lancet 1: 801 (1975).
36. JAMES, W. P. T., and SOUTHGATE, D. A. T. Bran and blood-lipids. Lancet 1: 800 (1975).
37. WELLS, A., and ERSHOFF, B. H. Beneficial effects of pectin in prevention of hypercholesterolemia and increase in liver cholesterol in cholesterol-fed rats. J. Nutr. 74: 87 (1961).
38. LEVEILLE, G. A., and SAUBERLICH, H. E. Mechanism of the cholesterol-depressing effect of pectin in the cholesterol-fed rats. J. Nutr. 88: 209 (1966).
39. SUNDARAVALLI, O. E., SHURPALEKAR, K. S., and NARAYANA RAO, M. Effects of dietary cellulose supplements on the body composition and cholesterol metabolism of albino rats. J. Agr. Food Chem. 19: 116 (1971).
40. SHURPALEKAR, K. S., DORAISWAMY, T. R., SUNDARAVALLI, O. E., and NARAYANA RAO, M. Effect of inclusion of cellulose in an atherogenic diet on the blood lipids of children. Nature 232: 554 (1971).
41. THIFFAULT, C., BELANGER, M., and POULIOT, M. Traitement de hyperlipoproteinemie essentielle de type II par un nouvel agent therapeutique, la celluline. Can. Med. Ass. J. 103: 165 (1970).
42. HOWARD, A. N., GRESHAM, G. A., and LINDGREEN, F. T. Lipoprotein studies on rats fed thrombogenic and atherogenic diets. J. Atheroscler. Res. 8: 739 (1968).
43. RANHOTRA, G. S. Effect of cellulose and wheat mill-fractions on plasma and liver cholesterol levels in cholesterol-fed rats. Cereal Chem. 50: 358 (1973).
44. LINDER, P., and MOLLER, B. Lignin: A cholesterol-lowering agent? Lancet 2: 1259 (1973).
45. HODGES, R. E., and KREHL, W. A. The role of carbohydrates in lipid metabolism. Amer. J. Clin. Nutr. 17: 334 (1965).
46. COHEN, A. M., TEITELBAUM, A., BALOGH, M., and GROEN, J. J. Effect of interchanging bread and sucrose as main source of carbohydrate in a low fat diet on the glucose tolerance curve of healthy volunteer subjects. Amer. J. Clin. Nutr. 19: 59 (1966).
47. GROEN, J. J., BALOGH, M., YARON, E., and FREEMAN, J. Influence of the nature of the fat in diet high in carbohydrate (mainly derived from bread) on the serum cholesterol. Amer. J. Clin. Nutr. 17: 296 (1965).
48. MATHUR, K. S., KHAN, M. A., and SHARMA, R. D. Hypercholesterolaemic effect of Bengal gram. A long-term study in man. Brit. Med. J. 1: 30 (1968).
49. TSAI, A. C., ROMSOS, D. R., and LEVEILLE, G. A. Effect of dietary cholesterol on hepatic lipogenesis and plasma insulin and free fatty acid levels in rats. J. Nutr. 105: 939 (1975).
50. ALBRINK, M. J. Triglyceridemia. J. Amer. Diet. Ass. 62: 626 (1973).
51. MUELLER, J. F. A dietary approach to coronary artery disease. J. Amer. Diet. Ass. 62: 613 (1973).
52. PURDOM, M. E., MONDRAGON, N. H., PRYOR, W. W., and GRACY, R. W. Effect of carbohydrates on growth, plasma proteins, and liver enzymes. J. Amer. Diet. Ass. 60: 394 (1972).

[Received July 21, 1975. Accepted October 10, 1975]