Effect of Feeding Fat Blends of Hydrogenated Groundnut (Peanut) Fat and Cottonseed Oil Containing Different Levels of Linoleic Acid on Serum Cholesterol Levels in Monkeys (*Macaca radiata*) and Liver Cholesterol Concentration in Cholesterol-Fed Rats

S. N. JAGANNATHAN
Nutrition Research Laboratories, Indian Council of Medical Research, Hyderabad-7, India

Long-term studies reported from these laboratories (Gopalan et al., '60a) on monkeys maintained with high-fat diets suggested an approximate inverse relationship between the polyunsaturated fatty acid (PUFA) content of the various dietary fats and the changes they brought about in serum cholesterol levels. Thus a diet containing high amounts of butter or hydrogenated vegetable fat, both of which are poor in PUFA content, resulted in a marked elevation of serum cholesterol levels in both human volunteers and monkeys (Gopalan and Ramanathan, '56, '58). It was also shown that addition of a fat rich in PUFA, such as nigerseed oil at a level of 5% to a diet already containing 25% of hydrogenated groundnut fat resulted in a significantly lower order of elevation in serum cholesterol than a diet containing a 25% hydrogenated groundnut fat alone (Gopalan et al., '60a).

These observations suggested the possibility of minimizing the hypercholesterolemic effect of fats like butter, hydrogenated groundnut fat and coconut oil by admixing them with PUFA. It should however be borne in mind that the main advantage of these fats is their keeping quality, and any indiscriminate addition of PUFA would seriously hamper this advantage. Hence the need to find out the minimal level of PUFA that should be admixed with a fat so that even a high consumption of it does not elevate serum cholesterol level considerably. The present study is an attempt at elucidating this aspect.

MATERIAL AND METHODS

Test fats. Refined, deodourized unhydrogenated cottonseed oil was blended with hydrogenated groundnut fat in the required proportions so as to provide linoleic acid contents of zero, 2.5, 5.0, 7.5 and 10.0% in the blend. Besides adjusting the blends to give the desired PUFA[1] (linoleic acid) contents, they were adjusted to give a melting point of 36°C.[2] The composition and some analytical characteristics of the test fats used are shown in table 1.

Experimental diets. The proportion in which the various fat blends were mixed with the other ingredients of the experimental diet for the monkeys is shown in table 2. The test fat was freshly included every day at a 30% level in the diet, supplying 50% of the total calories. The steam-cooked diets were supplied to the animals in two divided portions made available 4 hours apart as this type of "intermittent feeding" (Jagannathan and Gopalan, '60) has been shown to produce marked changes in serum cholesterol concentration (Gopalan et al., '60b). Fresh water was supplied ad libitum to the animals.

The composition of the diet used for the experiment on rats is given in table 3. The nonfat ingredients of the diet consisted mainly of foodstuffs normally consumed in India and were included in proportions approximating those found in

Received for publication January 17, 1962.

[1] By PUFA is meant in this study only linoleic acid.

[2] The author is grateful to Dr. G. S. Hattiangdi, Hindustan Lever Ltd., Bombay for the kind supply of the various fat blends used in the study.

TABLE 1

Composition and some analytical characteristics of the fat blends used in the study

Blend no.	1	2	3	4	5
Composition, (%)					
Hydrogenated groundnut fat, m.p. 34°C	85.0	79.4	—	60.2	52.0
Hydrogenated groundnut fat, m.p. 36.5°C	—	—	89.4	—	—
Hydrogenated groundnut fat, m.p. 43°C	15.0	16.0	—	24.0	27.0
Cottonseed oil	—	4.6	10.6	15.8	21.0
Melting point (°C)	36.0	36.1	35.0	36.3	36.2
Nominal linoleic acid content, %	0	2.5	5.0	7.5	10.0
Linoleic acid content as analyzed[1]	0.5	2.4	5.0	7.4	9.2

[1] The author is grateful to Dr. Hattiangdi of the Hindustan Lever Ltd., Bombay and Dr. M. R. Subbaram, Regional Research Laboratory, Hyderabad-9, for their help in independent checks of the linoleic acid content of the fat blends.

TABLE 2

Composition of diet for monkeys

Ingredients	Parts
Wheat flour	528
Casein	120
Fat	300
Salt mixture[1]	40
Vitamin mixture[2]	10
Choline chloride	2
Vitamin A 7000 IU/animal/day Vitamin C 50 mg/animal/day	Added daily to the cooked diet

[1] Contained in gm: calcium lactate, 390; calcium phosphate, 162; potassium phosphate, 183; sodium chloride, 52; magnesium sulphate, 80; sodium phosphate, 104; potassium iodide, 5; and ferrous sulphate, 35.

[2] Supplied, in mg/kg diet: thiamine·HCl, 10; riboflavin, 20; niacin, 100; pyridoxine·HCl, 20; calcium pantothenate, 40; p-aminobenzoic acid, 200; inositol, 200; and vitamin K, 20.

TABLE 3

Composition of the diet for the rats

Ingredients	Parts
Wheat flour	450
Red gram (*Cajanus cajan*)	150
Skim milk powder	100
Sugar	50
Fat	200
Salt mixture[1]	20
Vitamin mixture[2,3]	6
Choline-starch mixture[4]	4
Cholesterol	10

[1] Hawk and Oser ('31) as modified by Bliss and Gyorgy ('51).

[2] Supplied per kg of diet, the vitamins in the following amounts in mg: thiamine·HCl, 5; riboflavin, 8; niacin, 40; pyridoxine·HCl, 5; calcium pantothenate, 40; biotin, 0.4; folic acid, 2; menadione, 50; inositol, 100; and p-aminobenzoic acid, 100.

[3] Adequate amounts of vitamins A and D were fed orally twice a week.

[4] Fifty grams of choline choride ground well with 150 grams of starch amylum.

well-fed lacto-vegetarian Indian communities. Cholesterol was included at a 1% level. The respective test fats were included at a 20% level supplying about 45% of total calories. The diets after being steam-cooked for 20 minutes were offered to the animals in individual stainless steel cups.

Experiment with monkeys. Twenty-six adult male monkeys of the species *Macaca radiata* weighing between 3.2 and 6.4 kg were used in this investigation. The animals had been previously maintained with an adequate stock diet containing 8% of fat for 4 to 5 weeks before they were used for the investigation. Two determinations of serum cholesterol concentration were carried out in all the animals after this stabilization period. The monkeys were then divided into 5 dietary groups with nearly similar distribution of body weights and initial serum cholesterol levels.

The animals were fed diets containing at a 30% level, one or other of the different fat blends prepared as mentioned earlier. Group 1 had the hydrogenated groundnut fat with no added linoleic acid, as the source of dietary fat while groups 2 to 5 had the blends of hydrogenated groundnut fat plus unhydrogenated cottonseed oil containing 2.5, 5.0, 7.5 and 10.0% of linoleic acid, respectively. Blood was drawn from the animals at the end of feeding the respective diets for 4, 7 and 10 weeks, and serum cholesterol concentration was determined.

Experiment on rats. Young growing male albino rats were divided into 5 groups and were fed diets differing only in the fat

included. Groups 1 to 5 received the fat blends of linoleic acid content of zero, 2.5, 5.0, 7.5 and 10.0%, respectively. The diets were fed ad libitum and a record of food intake was kept. The animals were weighed weekly. At the end of 8 weeks the rats were sacrificed and determination of serum cholesterol, liver cholesterol and total liver lipids was made.

Analytical methods. Blood was drawn from the femoral vein of the monkeys and by cardiac puncture under ether anaesthesia in the rats. Serum cholesterol concentration was determined by the method of Abell et al. ('52). At the end of the experimental period, the rats were sacrificed, the liver was removed, freed from adhering blood by blotting with folds of filter paper, weighed and taken for estimation of cholesterol and total lipid concentration. The whole liver was ground with 5 times its weight of anhydrous sodium sulphate and a little glass powder and was transferred to a glass-stoppered bottle in which it was extracted repeatedly with fresh quantities of diethyl ether. The ether extracts were combined together and filtered to make to a volume of 250 ml.

Five-milliliter aliquots were evaporated in glass-stoppered test tubes, saponified with alcoholic potash, extracted with petroleum ether (40 to 60°C), suitable aliquots evaporated and the cholesterol content was estimated by the method of Abell et al. ('52).

For the determination of liver lipids, 20 ml of the ether extract were evaporated, the residue redissolved in petroleum ether (40 to 60°C) and filtered through an ether-washed fat-free filter paper into a previously weighed container. The filter was washed with distilled pertroleum ether and the combined filtrate evaporated in an atmosphere of nitrogen to dryness. The lipid residue was dried by heating it at 50°C *in vacuo* for one hour and weighed.

RESULTS

The pattern of changes in serum cholesterol concentration in groups of monkeys fed the various fat blends containing different levels of linoleic acid (LA) is shown in figure 1. To enable evaluation of the statistical significance of the influence of the various fats on serum cholesterol level, values are given in table 4 which indicate the maximal deviation from the basal low-fat period level, reached during a period of 10 weeks along with the standard error of the mean. The

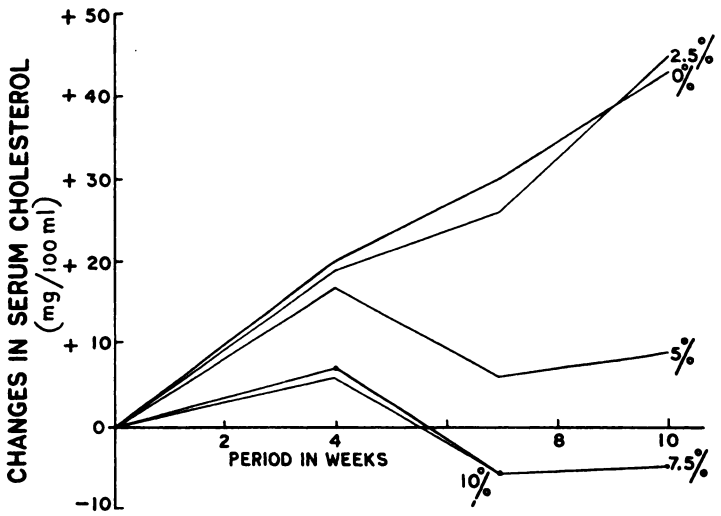

Fig. 1 Effect of feeding hydrogenated groundnut fat admixed with cottonseed oil to provide varying levels of linoleic acid on serum cholesterol concentrations in groups of monkeys. The percentages indicate the level of linoleic acid in the blend. The reference line of zero indicates the value with a low-fat basal diet. Each line represents the averages for the respective group. Number of animals in each group is indicated in table 4.

TABLE 4

Effect of feeding hydrogenated groundnut fat admixed with cottonseed oil to provide different levels of linoleic acid on serum cholesterol levels in monkeys

Group	Linoleic acid content of the blend	No. of animals	Initial level[1] of serum cholesterol	Deviation of serum cholesterol from the basal level	
				Maximum value during 10 week period	Mean of values for periods 4, 7 and 10 weeks
	%		*mg/100 ml*	*mg/100 ml*	*mg/100 ml*
1	0	7	139	+33.6±4.16[2]	+21.9±2.93
2	2.5	5	124	+45.2±6.36	+30.4±4.05
3	5.0	5	139	+19.8±9.05	+10.8±9.50
4	7.5	5	134	+ 7.2±9.85	− 1.2±8.78
5	10.0	4	124	+ 5.5±2.72	+ 0.3±2.84

Statistical significance of the differences

	Groups compared	Maximum deviation reached	Mean deviation for periods 4, 7 and 10 weeks
1	7.5 % LA × 0 % LA	$P < 0.05$	$P < 0.02$
2	7.5 % LA × 2.5 % LA	$P < 0.02$	$P < 0.02$
3	10.0 % LA × 0 % LA	$P < 0.01$	$P < 0.001$
4	10.0 % LA × 2.5 % LA	$P < 0.01$	$P < 0.01$

[1] After feeding low-fat diet for four weeks.
[2] Values represent the mean ± standard error.

data have also been indicated in another way, viz. as the mean of deviation in serum cholesterol from basal level at the end of 4, 7 and 10 weeks. The results of the experiment on rats are given in table 5.

The group of monkeys receiving a diet containing the hydrogenated groundnut fat with 0% of linoleic acid showed a significant elevation in serum cholesterol concentration (fig. 1, table 4). Inclusion of cottonseed oil with hydrogenated groundnut fat to provide linoleic acid at a level of 2.5% in the fat did not alter the serum cholesterol pattern obtained. In animals receiving the fat blend with 5% of LA, however, the increase in serum cholesterol levels was of a smaller order compared with groups with lower linoleic acid levels, but this value was still considerably higher than the basal low-fat period value. Fat blend with linoleic acid at levels 7.5 and 10% produced practically no change in serum cholesterol concentration from the basal level. This indicates that the increase in serum cholesterol concentration resulting from the high consumption of hydrogenated groundnut fat can be prevented by including cottonseed oil providing 7.5% of linoleic acid.

The results of the experiment with rats (table 5) showed that the concentration of cholesterol in liver in the group fed the 7.5% LA blend, was significantly ($P < 0.05$) lower than that in groups fed lower levels, viz. 2.5 and 5.0% LA. Liver cholesterol expressed as percentage of total liver lipids on diets containing 7.5 and 10.0% LA, was significantly lower than with diets containing 2.5% LA ($P < 0.01$) and 5.0% LA ($P < 0.05$). This observation appears complementary to the results obtained with monkeys with respect to the serum cholesterol behavior.

DISCUSSION

It is known that the rat is normally resistant to hypercholesterolemia unless its diet contains cholic acid also along with cholesterol. Since the diets used in the present study on rats did not include cholic acid, no significant degree of hypercholesterolemia was induced in these animals. This may explain the observation in the present investigation that the cholesterol-fed rats receiving the various fat blends did not show significant differences in their serum cholesterol levels, unlike the monkeys. However, the observation that

TABLE 5

Effect of feeding fat blends with different levels of linoleic acid on the accumulation of hepatic cholesterol in cholesterol-fed rats

Linoleic acid content of the fat blend	No. of rats (all males)	Gain in weight in 8 weeks	Liver weight	Liver weight/100 gm body weight	Serum cholesterol level	Total liver lipids	Liver cholesterol		
							Total	Per gm moist tissue	As % of total lipids
%		*gm*	*gm*	*gm*	*mg/100 ml*	*gm/100 gm*	*mg*	*mg*	
0	6	182.5 ± 8.56[1]	9.16 ± 0.276	3.77 ± 0.118	80.5 ± 5.08	8.78 ± 0.894	168.7 ± 24.22	18.26 ± 2.399	20.58 ± 1.138
2.5	6	180.5 ± 10.59	9.49 ± 0.320	3.97 ± 0.151	87.0 ± 10.25	10.42 ± 0.818	202.0 ± 26.31	21.40 ± 2.767	20.30 ± 1.512
5.0	6	179.0 ± 11.62	9.13 ± 0.589	3.80 ± 0.157	70.7 ± 7.20	9.95 ± 0.638	174.5 ± 17.58	19.54 ± 1.680	19.58 ± 2.083
7.5	7	183.9 ± 6.62	9.96 ± 0.496	4.03 ± 0.160	76.7 ± 5.64	9.58 ± 0.639	137.9 ± 16.45	13.80 ± 1.407	14.34 ± 0.914
10.0	7	188.9 ± 6.69	10.20 ± 0.189	4.10 ± 0.104	77.6 ± 6.33	10.49 ± 1.003	151.4 ± 28.25	14.57 ± 2.545	13.20 ± 1.399

[1] Values represent the mean ± standard error.

in the cholesterol-fed rats, the fat blend with 7.5% of linoleic acid content resulted in a significantly lower liver cholesterol concentration than the blends with lower levels of linoleic acid appears to run parallel with the finding of a significantly lower serum cholesterol concentration in monkeys fed the fat blends with 7.5% of linoleic acid. For this reason the observations on rats were included in this report. It is possible that if hypercholesterolemia had been induced in the rats by the addition of cholic acid to the diet, the pattern of results might have been different.

The observation in the investigation on monkeys that inclusion of cottonseed oil to provide 7.5% of linoleic acid in hydrogenated groundnut fat could nullify the hypercholesterolemia normally associated with a high consumption of the fat, is of practical significance. These results should be viewed in the light of the earlier report from these laboratories (Gopalan et al., '60a) in which a rough parallelism was shown between the polyunsaturated fatty acid (PUFA) content of the fat and the changes brought about in serum cholesterol level, supporting the observations of Kinsell et al. ('58). It was, however, pointed out that while a diet containing 25% of groundnut oil (PUFA content 28%) still increased serum cholesterol, although to a lower level as compared with other fats, a 25:5 mixture of hydrogenated groundnut fat (PUFA content 2%) and nigerseed oil (PUFA 73%) containing only about 14% PUFA produced a more or less similar response, thus possessing an influence on serum cholesterol out of proportion to its PUFA content. The observation in the present investigation that a fat blend of hydrogenated groundnut fat and cottonseed oil containing 7.5% PUFA (linoleic acid) could prevent the increase in serum cholesterol normally found with hydrogenated groundnut fat, also suggests that besides the polyunsaturated fatty acids there are other factors coming into play especially when mixtures of different types of fats are involved. This may also follow from the report of Hashim et al. ('59) that in hypercholesterolemic patients receiving 40% of calories derived from fat, equal mixtures of safflower oil and

coconut oil were at least as effective as safflower oil alone in lowering the serum cholesterol level. The suggestion of Portman and Stare ('59) that at certain levels of dietary fat, maximal lowering of the serum cholesterol level can be obtained at less than maximal concentration of PUFA in the dietary fat is interesting in this context.

In the investigation reported by Gopalan et al. ('60a) on monkeys fed the various dietary fats singly and without the addition of dietary cholesterol, changes in serum cholesterol levels showed a correlation only with the PUFA content of the dietary fat. In the present investigation also using mixtures of hydrogenated groundnut fat and cottonseed oil, there was a rough parallelism between the changes in serum cholesterol levels and the linoleic acid content of the dietary fat. This does not however rule out the possibility of factors other than linoleic acid in dietary fat influencing cholesterolemia. The results of an investigation of some of these other factors are reported separately (Jagannathan, '62).

SUMMARY

Unhydrogenated cottonseed oil blended with hydrogenated groundnut fat so as to provide linoleic acid levels of zero, 2.5, 5.0, 7.5 and 10.0% in the blend, was included in the diet of adult male monkeys at a 30% level, supplying 50% of total calories. Serum cholesterol levels were determined after the animals had received the diet for 4, 7 and 10 weeks. The fat blends were included at a 20% level in the diet of young growing male rats along with 1% of cholesterol. Liver cholesterol and total lipids were determined at the end of 8 weeks.

The results showed that the hypercholesterolemia normally associated with a high consumption of hydrogenated groundnut fat in monkeys, could be prevented by incorporating into the fat cottonseed oil providing linoleic acid at a level of 7.5%.

The concentration of liver cholesterol in cholesterol-fed rats maintained with the fat blend containing 7.5% of linoleic acid, was significantly lower than in those that were maintained with the blends having lower levels of linoleic acid.

ACKNOWLEDGMENTS

The author acknowledges with gratitude the valuable guidance he received from Dr. C. Gopalan, Director, Nutrition Research Laboratories, in the conduct of this investigation. He is also grateful to Dr. S. G. Srikantia for his helpful criticisms and valuable suggestions.

LITERATURE CITED

Abell, L. L., B. B. Levy, B. B. Brodie and F. E. Kendall 1952 A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. J. Biol. Chem., *195:* 357.

Bliss, C. I., and P. György 1951 The animal vitamin A assays. In Vitamin Methods, Vol. II. Academic Press, Inc., New York, p. 53.

Gopalan, C., and K. S. Ramanathan 1956 Fats and disease. Lancet, *2:* 1212.

——— 1958 Effect of some dietary factors on serum cholesterol. Indian J. Med. Res., *46:* 473.

Gopalan, C., S. G. Srikantia and K. S. Ramanathan 1960a Effect of different fats on serum cholesterol levels in monkeys. Indian J. Med. Res., *48:* 495.

Gopalan, C., S. G. Srikantia, S. N. Jagannathan and K. S. Ramanathan 1960b Effect of the mode of feeding of fats on serum cholesterol levels and plasma fibrinolytic activity of monkeys. Fifth Internatioal Congress on Nutrition, Washington, D. C., abstract no. 343.

Hashim, S. A., R. C. Clancy, D. M. Hegsted and F. J. Stare 1959 Effect of mixed fat formula feeding on serum cholesterol level in man. Am. J. Clin. Nutrition, *7:* 30.

Hawk, P. B., and B. L. Oser 1931 A modification of the Osborne-Mendel salt mixture, Science, *74:* 369.

Jagannathan, S. N. 1962 Effect of feeding different fat mixtures providing the same amount of linoleic acid on serum cholesterol levels in monkeys and liver cholesterol concentrations in cholesterol-fed rats. J. Nutrition, *77:* 323.

Jagannathan, S. N., and C. Gopalan 1960 Effect of different diets on plasma fibrinolytic activity in monkeys. Indian J. Med. Res., *48:* 756.

Kinsell, L. W., R. W. Friskey, S. D. Michaelis and S. Splitter 1958 Essential fatty acids, lipid metabolism and atherosclerosis. Lancet, *1:* 334.

Portman, O. W., and F. J. Stare 1959 Dietary regulation of serum cholesterol levels. Physiol. Revs., *39:* 407.