Further Observations on Plasma Lipids of Vegetable Oil Fed Monkeys.* (28421)

SACHCHIDANANDA BANERJEE and ASOK BANDYOPADHYAY
Department of Physiology, Sardar Patel Medical College, Bikaner, India

We have shown(1) that the presence of vegetable oils in the diet of Rhesus monkeys helped in absorption of synthetic cholesterol mixed with the diet, leading to hypercholesteremia. Saturation, unsaturation or fatty acid composition of the oils administered did not seem to be responsible for the plasma changes observed. It was of interest to study the effect of administration of different vegetable oils only, without supplementation of extraneous cholesterol, for a prolonged period, on plasma lipids of Rhesus monkeys. As in the previous studies(1) the oils were fed at 10% level. It was of interest also to see if increasing these oils to 20% level in the diet could further enhance the absorption of cholesterol. These studies are presented here.

Materials and methods. Nine female Rhesus monkeys averaging 4 kg in weight, were fed a basal diet and vitamins(1) for 2 months. After collecting fasting blood samples for determination of the plasma lipids, the animals were divided into groups of 3 animals each. Two animals of each group received the basal diet containing 20% of the oil, while the third animal received in addition 3 g cholesterol. Vegetable oils used were sesame oil, mustard oil and coconut oil. Iodine values and fatty acid composition of the oils have been reported(1).

Fasting blood samples were collected at monthly intervals, for 8 months after feeding the different oils, and plasma was used for estimations of total cholesterol, nonesterified fatty acids (NEFA), phospholipids, triglycerides, β-lipoprotein cholesterol, and lipoproteins(2). Plasma insulin-like activity was measured initially and after 8 months of feeding the oils(3).

Feces were collected for 7 consecutive days during each month of experiment, dried, powdered, and extracted in a Soxhlet apparatus. An aliquot of the ether extract was evaporated to dryness and fecal Lieberman-Burchard reacting sterols determined in the same manner as in plasma total cholesterol(2). Fecal lipids were determined gravimetrically from the ether extract. Fecal cholic acid and deoxycholic acid were extracted and estimated as described by Haust and Beveridge (4).

Results. Plasma cholesterol. Total plasma cholesterol rose from the initial level in all monkeys after the oils had been fed for a month. Plasma cholesterol began to diminish from the second month in monkeys fed sesame oil and mustard oil. The rise in plasma cholesterol continued up to third month in the coconut oil-fed monkeys, then began to diminish. Plasma cholesterol was below initial value in all animals at the end of the eighth month of feeding the oils. Plasma cholesterol of monkeys fed cholesterol along with sesame oil and coconut oil, continued to increase, became highest after 3 months, began to diminish from the fifth month and at the end of eight months the value remained higher than the initial value. The increase in plasma cholesterol persisted up to 6 months in the animal fed cholesterol with mustard oil but the overall increase was least marked. Maximum rise in plasma cholesterol was observed in the monkey fed cholesterol and sesame oil. *Plasma β-lipoprotein cholesterol* increased proportionately with plasma total cholesterol in the monkeys receiving oils and also in the animal which received cholesterol with mustard oil. The value in monkeys fed cholesterol with sesame oil or coconut oil was not consistent, remained high up to 6 months and diminished slightly from 7 months, although its proportion to plasma total cholesterol remained very high (Table I).

Fecal bile acids. Fecal excretion of cholic acid and deoxycholic acid increased in all monkeys when fed the different oils with or

* Aided by a grant from Council of Scientific & Industrial Research, New Delhi.

TABLE I. Total Plasma Cholesterol and β-Lipoprotein Cholesterol of Monkeys Fed Vegetable Oils (mg/100 ml).

Monkey No.	Oil fed	Months after oils were fed 0	1	2	3	5	6	7	8
20	Sesame oil	175 (92)	184 (115)	173 (63)	165 (107)	155 (100)	152 (66)	124 (39)	129 (56)
27	*Idem*	134 (73)	160 (73)	129 (63)	129 (50)	119 (43)	128 (43)	101 (39)	123 (50)
18	" + cholesterol	112 (76)	339 (230)	347 (170)	414 (170)	285 (205)	321 (237)	288 (178)	339 (212)
24	Mustard oil	187 (142)	212 (159)	186 (107)	160 (73)	165 (73)	170 (96)	159 (48)	160 (48)
25	*Idem*	114 (69)	158 (115)	120 (70)	110 (70)	111 (50)	112 (59)	103 (39)	105 (39)
15	" + cholesterol	129 (69)	212 (137)	280 (137)	285 (152)	300 (152)	285 (143)	218 (126)	218 (126)
22	Coconut oil	116 (69)	148 (90)	158 (75)	170 (80)	145 (75)	174 (113)	112 (39)	112 (39)
23	*Idem*	129 (82)	218 (167)	203 (107)	210 (160)	165 (79)	178 (105)	96 (39)	97 (39)
17	" + cholesterol	120 (76)	299 (173)	663 (370)	760 (320)	578 (412)	618 (495)	390 (236)	244 (202)

Figures in parentheses indicate values of β-lipoprotein cholesterol.

without the supplement of cholesterol during the first 1-3 months. Values were quite low at the end of 6 months and excretion diminished below the initial value at the end of 8 months in all animals except one which received cholesterol along with coconut oil, where bile acid excretion remained very high. The fluctuations in fecal excretion of bile acids in monkeys fed cholesterol along with oils could be correlated with fluctuations in plasma levels of cholesterol (Table II). *Fecal lipids and sterols.* Lipid content of feces increased during first 2 months of administration of oils and subsequently diminished. The oils were absorbed from the gut. Presence of cholesterol in addition to oils in the diet did not alter the lipid content of feces. Fecal excretion of sterols did not change considerably up to third month, began to decrease gradually from fifth month and the value went below the initial figure at the end of 8 months of feeding the oils. Monkeys fed cholesterol along with oils excreted an increased quantity of sterols in the feces after one month. The excretion subsequently diminished. There was an inverse relation between plasma cholesterol and fecal excretion of sterols (Table III). *Plasma phospholipids.* Plasma phospholipids increased in all the experimental groups of animals and the values were well above the initial levels at the end of 8 months. Monkeys fed cholesterol along

TABLE II. Fecal Excretion of Combined Cholic and Deoxycholic Acids in Monkeys Fed Vegetable Oils (mg/Day).

Monkey No.	Oil fed	Months after oils were fed 0	1	2	3	5	6	7	8
20	Sesame oil	90	205	102	119	100	91	78	77
27	*Idem*	90	208	175	143	98	80	80	79
18	" + cholesterol	104	137	214	86	137	32	—	32
24	Mustard oil	74	74	218	165	93	42	49	49
25	*Idem*	130	195	140	137	148	61	55	60
15	" + cholesterol	104	409	316	121	93	—	—	100
22	Coconut oil	120	214	225	230	220	84	71	72
23	*Idem*	76	241	—	221	170	68	69	69
17	" + cholesterol	61	140	131	84	221	64	—	204

TABLE III. Fecal Lipid (g/Day) and Fecal Lieberman-Burchard Reacting Sterols (mg/Day) of Monkeys Fed Vegetable Oils.

Monkey No.	Oil fed	Months after oils were fed 0	1	2	3	5	6	7	8
20	Sesame oil	.67 (62)	1.32 (74)	1.38 (67)	1.20 (96)	1.32 (41)	.78 (110)	.60 (60)	1.60 (30)
27	*Idem*	.60 (56)	3.28 (54)	1.86 (26)	1.01 (66)	1.02 (52)	1.56 (28)	1.50 (30)	1.50 (28)
18	" + cholesterol	.31 (40)	3.70 (2510)	2.65 (622)	1.84 (473)	2.19 (669)	1.12 (649)	.71 (134)	.70 (140)
24	Mustard oil	.84 (62)	.76 (24)	5.09 (103)	2.05 (49)	1.09 (30)	3.30 (63)	1.86 (30)	1.86 (30)
25	*Idem*	.69 (55)	1.73 (60)	3.65 (54)	1.71 (35)	1.12 (20)	2.86 (29)	1.76 (30)	1.10 (27)
15	" + cholesterol	.49 (115)	5.52 (1010)	6.17 (956)	2.82 (517)	2.38 (369)	2.12 (261)	1.10 (529)	2.76 (530)
22	Coconut oil	.44 (171)	5.89 (99)	3.72 (71)	1.53 (58)	2.42 —	1.07 (46)	.66 (29)	.66 (29)
23	*Idem*	.67 (66)	5.07 —	2.39 (58)	1.30 (50)	1.40 (43)	1.14 (41)	.69 (26)	.70 (27)
17	" + cholesterol	.35 (34)	6.10 (1065)	3.80 (643)	2.54 (605)	3.77 (771)	2.87 (561)	1.20 (160)	3.71 (385)

Figures in parentheses are fecal Lieberman-Burchard reacting sterols.

with coconut oil and sesame oil had higher plasma phospholipids than other monkeys (Table IV). *Plasma triglycerides.* Plasma triglycerides increased at one month, diminished in second month and came down to normal level at the fifth month after feeding of the oils. Monkeys which received cholesterol along with the oils showed higher levels of plasma triglycerides throughout the experiment. *Plasma NEFA.* There was an initial increase in plasma NEFA in all monkeys after they were fed oils but the value came down subsequently to normal range. Monkeys receiving cholesterol along with the oils had elevated plasma NEFA throughout the experimental period (Table V). *Plasma lipoproteins and insulin-like activity.* Plasma insulin-like activity and lipoproteins did not change after the monkeys were fed the different vegetable oils. When fed cholesterol along with the oils for 8 months the animals had diminished plasma insulin-like activity and increased plasma β-lipoprotein (Table VI).

Discussion. There had been a slight increase in total plasma cholesterol at the end of first month of feeding of the different oils. This seemed to be due to sudden change in fat content of diet from 2% to 20%. The administered fat was well absorbed as evidenced by the low fecal fat. The sudden increase of plasma triglycerides possibly fur-

TABLE IV. Plasma Phospholipids of Monkeys Fed Vegetable Oils (mg Lecithin/100 ml).

Monkey No.	Oil fed	Months after oils were fed 0	1	2	3	5	6	7	8
20	Sesame oil	105	267	328	335	250	220	233	173
27	*Idem*	197	267	357	231	220	218	158	142
18	" + cholesterol	98	364	393	391	328	343	328	280
24	Mustard oil	145	300	320	380	284	268	233	233
25	*Idem*	127	252	320	235	259	208	115	183
15	" + cholesterol	125	250	313	325	287	273	258	180
22	Coconut oil	185	270	325	335	241	213	258	250
23	*Idem*	127	280	328	353	240	250	233	233
17	" + cholesterol	98	492	535	525	465	418	345	272

TABLE V. Plasma Triglycerides (mg/100 ml) and Plasma NEFA (μeq/l) of Monkeys Fed Vegetable Oils.

Monkey No.	Oil fed	Months after oils were fed 0	1	3	5	8
20	Sesame oil	293 (48)	365 (80)	273 (61)	117 (58)	382 (63)
27	*Idem*	183 (68)	195 (82)	409 (46)	351 (53)	117 (54)
18	" + cholesterol	234 (43)	365 (121)	702 (106)	878 (65)	666 (276)
24	Mustard oil	273 (59)	352 (87)	272 (62)	410 (42)	176 (64)
25	*Idem*	351 (40)	440 (85)	312 (89)	293 (65)	255 (63)
15	" + cholesterol	351 (40)	365 (85)	390 (89)	936 (65)	714 (63)
22	Coconut oil	235 (40)	485 (79)	410 (54)	176 (62)	195 (62)
23	*Idem*	205 (41)	429 (79)	351 (66)	234 (44)	293 (42)
17	" + cholesterol	254 (41)	438 (109)	761 (240)	1170 (114)	689 (118)

Figures in parentheses are values for plasma triglycerides.

TABLE VI. Lipoproteins and Insulin-Like Activity of Plasma of Monkeys Fed Vegetable Oils.

Monkey No.	Oil fed	Lipoproteins (%) Initial α	Initial β	After 8 mo α	After 8 mo β	Insulin-like activity* Initial	After 8 mo
20	Sesame oil	42	58	36	64	11.81	8.45
27	*Idem*	50	50	41	59	9.97	7.57
18	" + cholesterol	40	60	11	89	4.33	no uptake
24	Mustard oil	46	54	50	50	7.56	8.00
25	*Idem*	41	59	47	53	2.00	1.46
15	" + cholesterol	46	54	13	87	21.70	2.47
22	Coconut oil	46	54	45	55	19.61	16.27
23	*Idem*	42	58	45	55	3.84	4.00
17	" + cholesterol	46	54	16	84	15.68	7.66

* mg glucose uptake/g dry rate diaphragm/ml plasma/90 min incubation.

nished more acetate to be utilized for synthesis of cholesterol. The body subsequently adapted itself to the increased fat intake, metabolized the fat normally and as a consequence plasma cholesterol came down to basal level. Prolonged feeding of oil seemed to depress cholesterol biosynthesis which possibly explained the lower plasma cholesterol at the end of seventh month of feeding the oils. This was true irrespective of the saturation or unsaturation of the oils. Hypercholesteremia, therefore, does not seem to depend on saturation or unsaturation of the fat consumed.

With the rise in plasma cholesterol there was increased fecal excretion of bile acids. With the decrease in plasma cholesterol, however, fecal bile acid excretion did not increase. An insignificant portion of cholesterol is degraded into bile acids and the proportion of cholesterol thus degraded might not change ordinarily. Mosbach *et al.*(5) observed that feeding bile acids to dogs resulted in decreased rates of conversion of cholesterol to bile acids. Diminished fecal excretion of bile acids in monkeys in the latter part of the experimental period might, therefore, be due to increased reabsorption of bile acids from the gut. Bronte-Stewart *et al.*(6), and Gordon *et al.*(7) reported that diminution in serum cholesterol in human subjects fed unsaturated fats was associated with increased excretion of bile acids. Our results in monkeys, however, do not support the above observation in human beings.

Cholesterol-fed monkeys receiving 20% fat in the diet had higher plasma cholesterol and excreted less sterols in the feces in comparison to the monkeys fed the oils at 10% level as described previously(1). This indicated that with the increase in dietary fat there was increased absorption of cholesterol. The catabolism of cholesterol as indicated by fecal bile acids was irregular in these animals. The increase in plasma β-lipoprotein cholesterol in monkeys fed cholesterol along with coconut oil or sesame oil was comparable with increase in plasma β-lipoproteins and plasma

total cholesterol. But in the monkey fed cholesterol and mustard oil, β-lipoprotein cholesterol did not increase with the increase in the plasma β-lipoproteins. As overall increase in plasma cholesterol in this monkey was least, cholesterol transported as β-lipoprotein was also low.

The increased plasma phospholipids in all monkeys fed oils, with or without cholesterol supplement, possibly indicated increased transport of fatty acids in combination with available phosphorus. The gradual diminution in plasma phospholipids in the latter part of the experimental period might indicate hepatic failure of phosphorylation of fatty acid. Monkeys which received cholesterol in addition to the oils had higher plasma phospholipid although there was no difference in absorption of oils as evidenced by fecal lipids. Increased plasma cholesterol might have some influence on level of plasma phospholipids.

Increased plasma NEFA in hypercholesteremic monkeys was probably due to diminished plasma insulin. This confirms our previous findings(3). Vegetable oils *per se* do not seem to influence the level of plasma NEFA.

Summary. Commonly used vegetable oils, sesame oil (iodine no. 110), mustard oil (iodine no. 104) and coconut oil (iodine no. 9), were fed to Rhesus monkeys at a 20% level in the diet for 8 months. Plasma lipids and fecal lipids were determined every month. Plasma insulin-like activity was measured before and at the end of eighth month. Some of the monkeys were also fed cholesterol along with the oils for 8 months. Plasma cholesterol of monkeys fed different oils did not change considerably during the experiment. There was a slight increase at the initial stage but it decreased below the basal values after 8 months of feeding the oils irrespective of their saturation or unsaturation. Plasma cholesterol of monkeys fed cholesterol along with the oils increased considerably but the increase was highest when sesame oil was fed. Plasma β-lipoprotein cholesterol increased in proportion to total plasma cholesterol after the oils were fed. After an initial increase, plasma triglycerides decreased to normal level at the end of fifth month of feeding the oils. The same was true for the plasma nonesterified fatty acids. There was an overall increase in plasma phospholipids in all monkeys. Monkeys fed cholesterol along with oils had increased NEFA values of plasma possibly due to diminished plasma insulin. Fecal total lipid, Lieberman-Burchard reacting sterols and bile acid excretion diminished gradually in all the monkeys. Fluctuations in plasma cholesterol in cholesterol-fed monkeys could be correlated with fecal excretion of sterols and bile acids.

1. Banerjee, S., Ghosh, P. K., Bandyopadhyay, A., PROC. SOC. EXP. BIOL. AND MED., 1962, v109, 313.

2. Banerjee, S., Bandyopadhyay, A., *ibid.*, 1963, v112, 372.

3. Bandyopadhyay, A., Banerjee, S., *ibid.*, 1962, v110, 173.

4. Haust, H. L., Beveridge, J. M. R., *Arch. Biochem. Biophy.*, 1958, v78, 367.

5. Mosbach, E. H., Abell, L. L., Halpern, E., *Circulation,* 1958, v18, 480.

6. Bronte-Stewart, B., Antonis, A., Eales, L., Brock, J. F., *Lancet,* 1956, v1, 521.

7. Gordon, H., Lewis, B., Eales, L., Brock, J. F., *ibid.*, 1957, v2, 1299.

Received March 28, 1963. P.S.E.B.M., 1963, v113.