EFFECT OF THE LEVEL AND TYPE OF DIETARY FAT ON THE METABOLISM OF CHOLESTEROL AND BETA LIPOPROTEINS IN THE CEBUS MONKEY*

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The new world monkey, *Cebus fatuella*, develops hypercholesteremia, hyperbetalipoproteinemia, and atherosclerosis when fed a diet containing alpha protein, 5 per cent cholesterol, and approximately 30 per cent of the supplied calories as corn oil (1). Elevations of serum lipide levels and vascular lesions of similar type and severity were also produced in the *Cebus* monkey by feeding diets containing casein, cholic acid, and cholesterol (2). Recently Mann (3) has shown that elevations of serum cholesterol and beta lipoproteins equivalent to those obtained by feeding diets containing alpha protein and 5 per cent cholesterol can be obtained when the dietary cholesterol is reduced to a 0.5 per cent level. The equivalence of vascular lesions established by feeding the 0.5 per cent and 5.0 per cent levels of cholesterol was not investigated.

The present work was undertaken to investigate the effects of the level and type of dietary fat on serum cholesterol and beta lipoprotein responses of the *cebus* monkey. A rather large number of studies in the past has tended to show a correlation between serum cholesterol levels and the levels of dietary fat in the human (4). Recently, however, reports from the laboratories of Groen (5) Kinsell (6), and Ahrens (7) have maintained that certain vegetable fats have a hypocholesteremic effect on humans. Malmros and Wigand (8) found that patients who have a hypocholesteremic response to dietary corn oil have this response obliterated when margarine is included in the diet. Other reports have given strength to the hypothesis that the relationship between serum cholesterol values and the type of dietary fat in humans depends primarily on

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the degree of saturation of the constituent fatty acids rather than on the origin of the fat (9, 10).

Several experimental approaches have indicated a relationship between cholesterol and fatty acid metabolism. Among these are the characterization of pancreatic cholesterol esterase (11) and lipoprotein lipase (12), and studies relating essential fatty acid deficiency syndrome in rats with the level of exogenous cholesterol (13).

EXPERIMENTAL

A total of 26 monkeys weighing 900 to 1350 gm. at the beginning of the experiment were used. The daily ration fed to all monkeys on a given experiment supplied equal amounts of calories, alpha protein,[1] cholesterol,[2] and trace supplements. This was accomplished by varying the level of sucrose when the level of dietary fat was varied and adjusting the weight of the other nutrients to a constant relationship to caloric value of the diet. Cholesterol was fed at the rate of 0.1 gm., protein 4.3 gm., minerals (14) 0.8 gm., inositol 0.02 gm., choline 0.1 gm., and oleum percomorphum 0.4 gtts. per 100 calories of diet. A daily supplement of B vitamins and ascorbic acid as previously described (1) was mixed with the ration. Animals were weighed weekly and there was no significant difference in mean weight change for monkeys of each experiment. When diets containing alpha protein were fed, there was minimal or no weight gain. When diets containing casein were fed, monkeys gained up to 30 gm./week.

Monkeys were bled by venipuncture at two week intervals for serum cholesterol and hemoglobin determinations and at 8 week periods for serum beta lipoprotein determinations. Serum total cholesterols were determined by the method of Abell *et al.* (15). In a few instances free and total cholesterols in the serum were determined by the method of Sperry and Webb (16). Serum beta lipoproteins were determined by the method of Gofman *et al.* (17).

The standard assay period was 8 weeks. It has been observed on the diets tested that the serum cholesterol values tend to reach a plateau value within 6 weeks. Frequently monkeys were used for several assays. The animals were placed on basal diets (casein-containing cholesterol-free) for 6 weeks between assays when it was desired to begin the next assay from basal serum cholesterol levels. There is considerable evidence that the cholesteremic response resulting during a second feeding period is similar to the previous response, although this point has not been rigorously proved.

RESULTS

Effect of Dietary Levels of Corn Oil.

Twelve monkeys were used in the first experiment. Their mean serum cholesterol level on a casein-containing, cholesterol-free diet was 135 mg. per cent. The animals were divided into 3 groups of 4 monkeys each. One group received 10 per cent, the second 32 per cent, and the third 45 per cent of the dietary calories as corn oil,[3] at the level of dietary cholesterol indicated above.

[1] Alpha protein was furnished through the courtesy of Mr. W. M. Bain, Glidden Co., Chicago.

[2] Crystalline cholesterol was furnished through the courtesy of Armour and Company, Chicago.

[3] Mazola (Corn Products Refining Company). Manufacturer's analysis of this product is quoted by Ahrens and Borgström (18).

The serum cholesterol values rose in all groups and appeared to reach a plateau at 4 or 6 weeks. The mean serum cholesterol and S_f12 to 100 beta lipoprotein values are indicated in Table I. The serum cholesterol values were higher in the monkeys fed the high than in those fed the low fat diets (396 mg. per cent compared to 260 mg. per cent, $p < 0.01$). The mean serum cholesterol values were also higher in the medium fat group (382 mg. per cent, $p < 0.05$). There is no statistical significance to the difference between the medium and high fat groups. The mean values for S_f12 to 100 beta lipoproteins were roughly proportional to the dietary level of fat, although there was great variability within groups. It should be remembered that the sera of *Cebus* monkeys which were fed cholesterol-free, casein-containing diets were almost uniformly free of S_f12 to 100 beta lipoproteins. The percentage of serum cholesterol in the free form was not affected by the level of dietary fat.

TABLE I

*The Effect of the Level of Dietary Fat (Corn Oil) on the Serum Cholesterol and Beta Lipoprotein Responses of Cebus Monkeys in 8 Weeks on a Hypercholesteremic Regimen**

Calories as corn oil	No. of monkeys	Serum cholesterol	Serum beta lipoproteins S_f 12 to 100
per cent		*mg. per cent*	*mg. per cent*
10	4	260 ± 15‡	77 ± 35
32	4	382 ± 43	123 ± 46
45	4	396 ± 34	201 ± 43

* See text for composition of diets.

‡ All values are expressed as means ± the standard errors of the means. Serum lipide determinations were made every 2 weeks. The 8 week determinations usually represented a plateau value.

At the end of 8 weeks of feeding the experimental diet the monkeys fed the 45 per cent fat diet were changed to the 10 per cent fat diet and *vice versa*. At the end of a second 8 week period some of the monkeys were again crossed. An increase in the dietary corn oil resulted in an increase in serum cholesterol levels while a decrease in dietary corn oil resulted in a decrease in serum cholesterol levels in all cases.

Effect of Dietary Carbohydrate on Serum Cholesterol Responses.—Since we have reported that the feeding of diets containing sucrose increased the degree of cholate-induced hypercholesteremia in rats above that of controls fed corn starch (19, 20), and since the feeding of fats at differing levels required variation of the levels of dietary carbohydrate, the effect of feeding sucrose- and starch-containing diets to the *Cebus* monkey was explored. The mean values for the serum total cholesterol at the end of 8 weeks on hypercholesteremic diets including sucrose and starch are shown in Table II. Also shown are the values

after reversing the diet for a second 8 week experimental period. The monkeys fed sucrose-containing diets had only slightly and not statistically greater serum cholesterol responses. Since the greater amounts of sucrose were fed to animals on the lower fat diets, the carbohydrate effect would be expected to minimize rather than accentuate the difference in serum cholesterol levels between animals fed high fat and those fed low fat diets.

Effect of Changing from Dietary Corn Oil to Hydrogenated Cottonseed Oil.

Ten monkeys which had been fed the hypercholesteremic diet (including alpha protein and cholesterol) with corn oil for 8 weeks or longer were changed to identical diets except for the substitution of a hydrogenated cottonseed oil[4] for corn oil. Four animals received 10 per cent, 3 received 32 per cent, and 3 received 45 per cent of the calories as fat.

The serum cholesterol and S_f12 to 100 beta lipoproteins of every monkey except one (No. 79) in the 32 per cent fat group rose when the change in dietary

TABLE II

*The Effect of the Type of Dietary Carbohydrate on Serum Cholesterol Responses of Cebus Monkeys in 8 Weeks on a Hypercholesteremic Regimen**

Dietary carbohydrate	No. of animals	Mean serum cholesterol	Changed to	Mean serum cholesterol
		mg. per cent		*mg. per cent*
Sucrose	4	382	Starch	336
Starch	4	369	Sucrose	404

* See text for composition of diets.

fat was made (Table III). The monkeys which were fed the 10 per cent fat diets had the greatest percentage increase in serum cholesterol and beta lipoproteins, although the absolute values were still lower than those of monkeys in the higher fat groups. The elevations indicated were, in general, obtained within 4 weeks and were maintained throughout observation periods of up to 20 weeks.

A single monkey, which was fed for 6 months on a diet including 0.1 gm. cholesterol per 100 calories of diet with 45 per cent of calories supplied as a hydrogenated cottonseed oil and in which serum cholesterol values were greater than 700 mg. per cent, was sacrificed, and the heart and great vessels were evaluated by Dr. Stephen Andrus of our department. Sudanophilic areas were observed, on the intimal surface; these were of the type described (1) in animals receiving approximately ten times the daily amount of cholesterol fed in the present experiments.

The Cholesteremic Response to Corn Oil and Hudrogenated Cottonseed Oil Containing Diets Without Cholesterol.—A preliminary study was made of the effect of feeding 45 per cent of the dietary calories as corn oil and as hydrogenated

[4] The cottonseed oils used in these experiments were furnished through the courtesy of Swift and Co., Chicago.

cottonseed oil in diets based on alpha protein and free of cholesterol. The cholesteremic responses of 3 monkeys fed the corn oil and 3 fed the hydrogenated cottonseed oil diets are indicated in Fig. 1. The animals fed the diets containing corn oil had no or minimal increases in serum cholesterol values above those of the control period (diets based on casein) during the first 6 weeks, whereas the monkeys fed the diets containing hydrogenated cottonseed oil had increases of 50 mg. per cent or better. At the end of 8 weeks on the experimental diets the pattern became less consistent.

TABLE III

Variations in the Levels of Serum Cholesterol and Beta Lipoproteins Resulting from Changing the Dietary Fat from Corn Oil to Hydrogenated Cottonseed Oil

Cholesterol supplied at the rate of 0.1 gm./100 calories of diet.

Monkey No.	Calories from fat	Serum cholesterol		Beta lipoproteins S_f 12 to 100	
		Corn oil	Hydrogenated cottonseed oil	Corn oil	Hydrogenated cottonseed oil
	per cent	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*
7-1	10	198	335	5	60
1-03	10	300	528	55	650
1-07	10	219	520	179	370
1-18	10	289	569	20	140
7-9	32	438	409	81	62
9-5	32	643	720	490	784
1-08	32	472	556	200	319
8-0	45	364	644	30	100
1-06	45	500	728	379	680
1-21	45	474	627	390	190

Effect of Changing from a Hydrogenated to an Unhydrogenated Cottonseed Oil.

Three monkeys which had been fed diets including 45 per cent of the dietary calories as hydrogenated cottonseed oil[4] were changed to a diet containing 45 per cent of the calories as an unhydrogenated cottonseed oil from the same lot. The diet was based on alpha protein and cholesterol at concentrations described above.

A comparison of the analyses of these two fats is indicated in Table IV. The changes in serum cholesterol are indicated in Fig. 2. Two monkeys responded to the unhydrogenated cottonseed oil diets with a lowering of serum cholesterol levels. The third monkey performed atypically in that serum cholesterol values were dropping before the diet change, continued to drop after the change, and then rose.

Comparison of the Cholesteremic Responses of Monkeys Fed Diets Containing Corn Oil and Lard.

Three monkeys were fed diets containing 45% of the dietary calories as corn oil and 3 were fed 45% of the calories as lard in diets including alpha protein and cholesterol for periods of

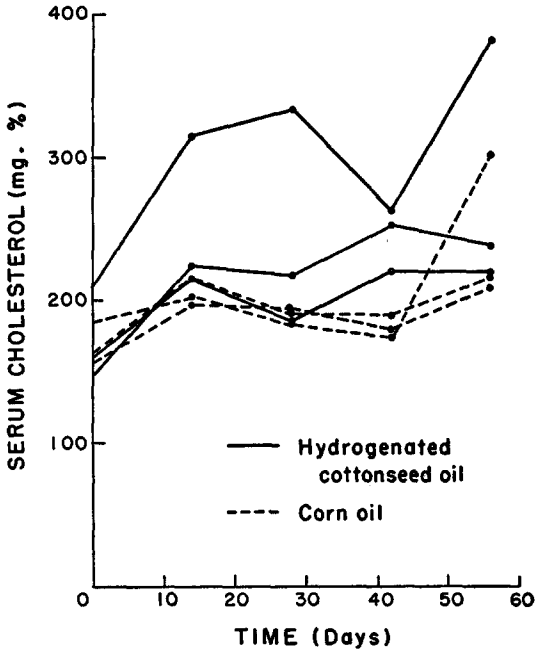

FIG. 1. The effect of feeding alpha protein diets containing corn oil and hydrogenated cottonseed oil, but without cholesterol, on the serum cholesterol responses of *Cebus* monkeys. See text for diet compositions. The dietary fat furnished 45 per cent of the calories.

TABLE IV

*Analyses of the Hydrogenated and Unhydrogenated Cottonseed Oils Used in These Experiments**

	Cottonseed oil	Hydrogenated oil
Iodine No.	108.4	70.9
Melting point	61°	100°
Linoleic acid	31.5 per cent	3.5 per cent
Linolenic acid	0.33	0.07
Arachidonic acid	0.00	0.00
Conjugated diene	0.32	1.01

* Analyses furnished through the courtesy of the laboratories of Swift and Company.

eight weeks. The cholesterol content of the lard was subtracted from the amount of crystalline cholesterol supplied to lard containing diets. At the end of eight weeks the diet groups were crossed and the experiment continued for 8 more weeks.

The serum cholesterol and S_f 12 to 100 beta lipoprotein responses are indicated in Fig. 3. The serum cholesterol and beta lipoprotein responses obtained

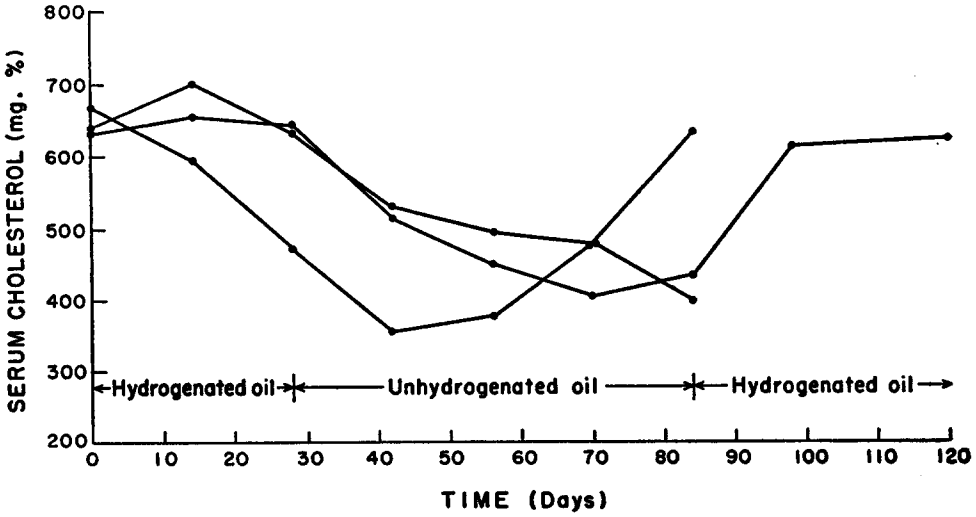

FIG. 2. The effect of feeding a hydrogenated and unhydrogenated cottonseed oil in diets containing cholesterol (0.1 gm./100 calories of diet) on the serum cholesterol responses of *Cebus* monkeys. The dietary fat furnished 45 per cent of the calories.

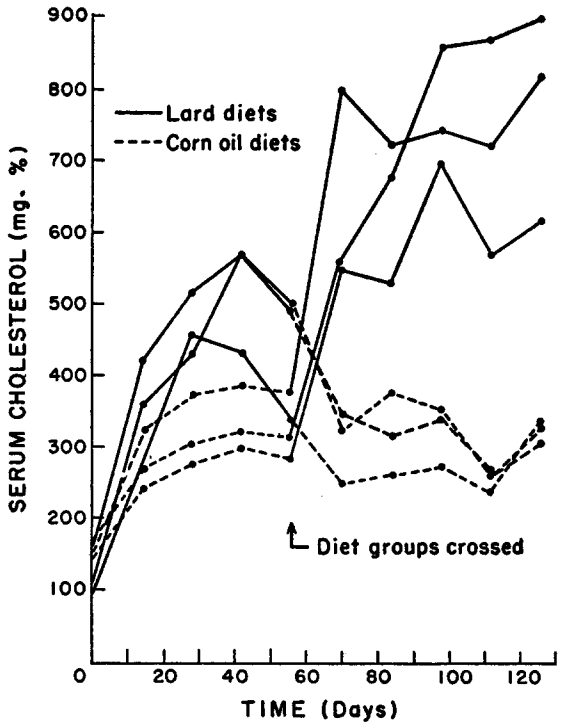

FIG. 3. The effect of feeding corn oil and lard containing diets (cholesterol = 0.1 gm./100 calories of diet) on the serum cholesterol responses of *Cebus* monkeys. The dietary fat furnished 45 per cent of the calories. At the end of 56 days the mean S_f 12 to 100 beta lipoproteins for monkeys on the lard diet was 180 mg. per cent and for the corn oil diet 80 mg. per cent. At the end of 112 days the mean S_f 12 to 100 beta lipoprotein values were: lard diets, 324 mg. per cent; corn oil diet, 52 mg. per cent.

with the lard diets were markedly greater than those obtained with the corn oil diets.

An atypical serum cholesterol response was observed in the first 8 week period. The monkeys fed the lard containing diets showed an initial rise in serum cholesterol levels and then a drop which was apparently continuing at the time of the first diet change. The significance of this drop cannot be explained.

The Effect of Adding Tung Oil to the Corn Oil-Containing Diets.—The analyses of hydrogenated cottonseed oil (Table IV) indicated that this fat had a higher concentration of fatty acids with the conjugated diene grouping than did the fat before hydrogenation. Furthermore, Brice *et al.* (21) have observed the presence of small levels of conjugated diene fatty acids in lards (0.23 per cent), tallows, and tallow soaps. One of us has shown that massive elevations of serum cholesterol can be induced in rats on diets only mildly hypercholesteremic when corn oil is the dietary fat, by the use of tung oil, which has a fatty acid composition very high in eleostearic acid, a conjugated trienoic fatty acid. It was, therefore, decided to add small amounts of tung oil[5] (2 ml./day), as a prototype of the conjugated double bond grouping, to the diets of animals receiving 45 per cent of their calories as corn oil. A supplement of tung oil equivalent to about one-tenth of the total fat of the ration, tested on 4 monkeys, had no effect on serum cholesterol or beta lipoprotein response to the high fat diet.

DISCUSSION

The data reported indicate that the hypercholesteremia and hyperbetalipoproteinemia, associated with the feeding of alpha protein and cholesterol at the rate of 0.1 gm./100 calories of diet to *Cebus* monkeys, is intensified by feeding higher levels of corn oil. An even more striking intensification is obtained by feeding fats that are naturally or artificially more saturated. Since only 4 types of fat were considered, it is difficult to ascribe the results to a single factor such as the iodine number, *per se*. Approximate iodine numbers for the 4 fats considered are: corn oil-120, cottonseed oil-108, hydrogenated cottonseed oil-71, and lard-58. The linoleic acid content of these fats is roughly proportional to the iodine number except that the linoleic acid content of most commercial lards (analyses not performed on the lard used here) is, at least, twice that of the hydrogenated cottonseed oil used in these experiments (3.5 per cent). The linoleic acid content of lard is very dependent upon the prior diet of the swine from which the lard is derived (22).

The process of hydrogenation results in a complex series of changes (23) in fatty acid moieties. Not only are unsaturated fatty acids, including linoleic,

[5] The tung oil used in this experiment was furnished through the courtesy of Swift and Co., Chicago.

largely reduced, but stereoisomers such as cis-trans and trans-trans forms not usually characteristic of natural fats are formed. The hydrogenated fat under consideration in these studies had a higher assay of conjugated diene than did the unhydrogenated original product. Nevertheless, the addition of tung oil which contains a conjugated trienoic fatty acid to diets containing corn oil did not appear to affect the serum cholesterol levels.

A difference in cholesterol absorption related to the level and type of fat, perhaps, associated with different rates of cholesterol esterification is possible, since different rates of cholesterol esterification with fatty acids of differing degrees of unsaturation have been observed (24). Alfin-Slater *et al.* (25) failed to note any difference in hepatic synthesis of cholesterol between rats fed different levels of cottonseed oil. They did present evidence that a high cottonseed oil diet resulted in a more rapid removal of cholesterol from the liver of rats than did a low cottonseed oil diet. It has been observed, however, that hepatic cholesterolosis is not characteristically associated with hypercholesteremia in the *Cebus* monkey (1).

The data suggest that monkeys fed on diets based on alpha protein and high levels of corn oil or hydrogenated cottonseed oil, but without cholesterol, develop elevations in serum cholesterol of a mild degree compared to control values obtained on a cholesterol-free casein-containing diet. Preliminary work from our laboratory (26) indicates that although the serum cholesterol of male rats is greater when cholesterol is fed with hydrogenated cottonseed oil as compared to unhydrogenated cottonseed oil, the serum cholesterols are higher in the group fed the unhydrogenated oil diets without dietary cholesterol.

Although Mann *et al.* have shown that the feeding of 5 per cent cholesterol to *Cebus* monkeys supplied diets containing casein and corn oil produces minimal elevations of serum cholesterol (about 250 mg. per cent), and we have obtained similar minimal elevations by feeding 0.5 per cent cholesterol, our findings indicate that serum cholesterol values of 500 to 800 mg. per cent can be obtained by feeding diets at the lower level of cholesterol with casein and hydrogenated cottonseed oil. The feeding of this latter diet results in striking weight gain, associated with the accumulation of large amounts of subcutaneous fat on the limbs and body.

Important limitations that can be placed on the data here presented result from the variability of monkeys with respect to age, weight, and other characteristics, the difficulty of maintaining accurate isocaloric feeding, and the instability of dietary lipides, particularly the polyunsaturated fatty acids.

In view of the findings of others (5–10) that high levels of unsaturated oils in various types of diets cause reduction in serum cholesterol concentrations and the speculative comments of Sinclair (27) on the essential unsaturated fatty acids, it is of interest to point out that an elevation of serum cholesterol

was readily produced in these studies when corn oil was the only source of fat. Further, the hypercholesteremia was accentuated when the corn oil was increased from 10 to 45 per cent of the dietary calories.

SUMMARY

A study was carried out to determine the effect of the level and type of dietary fat on the concentration of cholesterol and beta lipoproteins in the sera of *Cebus* monkeys. Three groups of monkeys were fed isocaloric diets containing a fixed ratio of alpha protein and cholesterol to calories but with different amounts of corn oil and sucrose. Corn oil provided 10, 32, and 45 per cent of the calories in the three diets, and the level of sucrose was varied inversely. After 8 weeks the serum cholesterol and S_f 12 to 100 beta lipoprotein concentrations were significantly greater in the medium and high fat groups. When corn oil was decreased from 45 to 10 per cent of dietary calories and sucrose was increased, the serum cholesterol fell in all cases, and when the reverse change was made, the concentration of serum cholesterol increased.

Variation in dietary sucrose had no specific effect. Substitution of starch for sucrose with diets otherwise constant did not cause significant change in the concentration of serum cholesterol.

When monkeys fed corn oil diets at any of three levels were changed to hydrogenated cottonseed oil diets at the same level, the serum cholesterol and S_f 12 to 100 beta lipoproteins rose. However, hydrogenated cottonseed oil had no greater hypercholesteremic effect than did corn oil in the absence of dietary cholesterol. Diets containing lard with cholesterol also produced strikingly greater serum lipide responses than did diets based on corn oil and cholesterol. Hydrogenated cottonseed oil had a greater hypercholesteremic effect than an unhydrogenated cottonseed oil from the same lot.

Preliminary studies indicated that the saturated fats (hydrogenated cottonseed oil) produced the most striking elevation of serum cholesterol values (above controls fed corn oil) when casein was the dietary protein.

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