Effect of the Saturation of Fats Upon the Disposition of Ingested Cholesterol

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A MASS of experimental data have accumulated that may be used as evidence that serum cholesterol levels are lowered when appreciable quantities of highly unsaturated fats are ingested.[1,17] This great interest has been stimulated largely as a result of the possible association of high blood cholesterol and lipid levels with the incidence of atherosclerosis. Much effort has also been expended in an attempt to determine the mechanism associated with changes in the blood cholesterol level as related to the degree of saturation of ingested fat. Studies have been made which suggest that the decrease in the serum cholesterol level may be the result of an increase in the excretion of bile salts,[2] of neutral sterols,[3] or an increase in deposition in the tissues.[4]

However, Gordon[1] states "that while there is no doubt that the consumption of certain unsaturated fats will lower the serum cholesterol level, the factors in these fats which determines their activity is at present unknown." The data of this investigation appear to support another interpretation of these changes in blood levels. Lowered levels after ingestion of either an unsaturated fat or a low fat diet could well be the result of a lack of an excess of saturated fats which are most effective in elevating the serum cholesterol level.

Conflicting reports may be the result of the many variables involved, such as the different experimental conditions or different species of animals employed. Therefore, it was suggested that a study be undertaken in an attempt to eliminate as many variables as possible. A balance study was designed to follow the known disposition of ingested cholesterol as a means of gaining further information about the mechanisms involved in altering blood cholesterol level by the ingestion of saturated and unsaturated fats. In this study similar animals were utilized as well as the same basic diet, time periods and methods of measurement. An attempt was also made to account for all the sterol ingested during a four week period in which the diet was supplemented with lard or corn oil, with and without added cholesterol and choline. In addition the final disposition of the radioactivity was followed after single feedings of labeled cholesterol to animals on similar diets.

MATERIALS

In the first experiment, male rats of the Sprague-Dawley strain, weighing approximately 200 gm., were divided into seven groups of seven animals each. Blood was obtained from each rat after an overnight fast for determination of the initial serum cholesterol values. On alternate days, five rats were started on the various special diets, one was kept on stock food and another was sacrificed in order to obtain a measure of carcass cholesterol content at the start of the experiment. The special diets fed were prepared by supplementing ground stock food pellets so that the diets contained 30 per cent lard or corn oil, with and without 3 per cent cholesterol. To one diet containing similar amounts

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TABLE I

Disposition of Cholesterol During a Four Week Period When Lard or Corn Oil Was Ingested With and Without Added Cholesterol and Choline

Dietary Supplements*	Ingested Sterols (gm.)	Fecal Sterols		Bile Acids‡ (gm.)	Carcass Sterols Gained (gm.)	Total Sterols Recovered (gm.)
		α-form (gm.)	β-form† (gm.)			
Lard	0.69	...	1.40	1.97	0.09	3.46
Corn oil	1.35	...	2.66	1.52	0.17	4.35
Lard plus cholesterol	15.57	2.71	4.87	3.51	0.84	11.93
Corn oil plus cholesterol	12.16	2.29	3.28	3.35	0.84	9.76
Lard plus cholesterol and choline	14.52	2.56	4.88	3.62	0.72	11.78

* The amounts of the supplements given were 30 per cent of the fat, 3 per cent cholesterol and 1 per cent choline.
† Only total sterols were determined in two groups, listed in second column.
‡ Values are expressed in equivalent weights of cholesterol.

of lard and cholesterol was added 1 per cent of choline.

Food intakes were followed and fecal collections made daily for each rat. The pooled individual fecal collections were kept at 5°c., then dried at 50°c. under reduced pressure, and finally weighed and powdered for sampling. Blood was drawn for cholesterol determinations and the rats were sacrificed at the end of four weeks on the experimental diets. After clipping the hair, the entire carcasses with viscera were weighed and finely ground. The tissues were minced in a Waring blendor in order to obtain more uniform aliquots and immediately frozen for analysis later.

In the second experiment, the rats were divided into three groups consisting of three rats of matched weights in each group. The rats in the first two groups were fed diets similar to those in the first experiment except that 2 per cent cholesterol and 0.4 per cent sodium taurocholate were added to the diets containing 30 per cent lard or corn oil. Those in the third group received only the unsupplemented stock diet.

After three weeks, the rats were weighed, fasted overnight, and given by stomach tube 30 μc. of cholesterol-4-C^{14} dissolved in 0.3 ml. of the dietary fat followed by 4 ml. of 0.5 per cent aqueous solution of the bile salt. After immediate return to the special diets, blood samples were taken from each rat at eight and twenty-four hours for determination of radioactivity. As previouly described, daily fecal collections were made on each rat during the terminal fourth week. The rats were then sacrificed by exsanguination and the liver removed. The livers and carcasses were finely ground separately and frozen for later analysis.

METHODS

Serum cholesterol values were determined according to the method of Abell et al.[5] Similarly, 0.5 gm. samples of tissue or feces were treated with alcoholic potassium hydroxide and the sterols extracted with petroleum ether. After isolation, the tissue and dietary sterols were determined by this method while the fecal sterols were measured by that of Zlatkis et al.[6] When desired, the digitonin precipitable sterols or β-sterols were separated by the method of Sperry and Webb[7] and determined according to the procedure of Feichtmeir and Bergerman.[8] The remaining sterol (α) was determined by difference between the total and β-sterol content. After removal of the sterols and the other lipids, the bile acids were separated and purified in a manner similar to that described by Siperstein and Chaikoff.[9] An adaptation of the technic of Bragdon[10] was utilized for the determination of the bile acids, using cholic acid as a standard. Radioactivity was determined in the second experiment, as previously described,[11] by direct mounting of the eight and twenty-four hour blood samples and by dry combustion of extractions of all others.

RESULTS

The final disposition of all sterols ingested during a four week period on the various diets in the first experiment is recorded in absolute amounts in Table I. Although the sterol intakes are quite different in the first two and last three groups, an almost identical increase in the weight of rats in all groups during the period would indicate a similar caloric intake by each of the five groups of rats.

TABLE II
Effect of Diets on Sterols of Blood, Carcass and Feces

Dietary Supplements	Blood Cholesterol		Carcass Sterols per 100 gm.		Total Fecal Sterols Recovered	
	Increase During Period (mg. %)	Increase in Level (%)	Final Value (mg. %)	Change from Initial Value* (%)	α-form (%)	β-form (%)
None	5	8	215†	100	...	...
Lard	13	19	160	74	...	...
Corn oil	6	8	186	86	...	...
Lard plus cholesterol	21	30	363	169	36	64
Corn oil plus cholesterol	12	16	389	181	41	59
Lard plus cholesterol and choline	24	36	337	157	34	66

* Comparisons with initial value set at 100 per cent.
† Initial value of controls sacrificed at beginning of study and final value were the same.

The effect of the various diets on blood cholesterol levels and tissue sterols per 100 gm. of carcass, along with the forms of recovered fecal sterols, are shown in Table II. As previously reported, blood cholesterol levels were definitely higher in the animals on similar diets which contained lard instead of corn oil. The levels were higher still when cholesterol was added to the diets, but the greatest elevation occurred when the diets contained the more saturated fat. The addition of choline to the diet apparently had no effect on blood cholesterol levels.

In both groups of rats on the high fat diets without added cholesterol the sterol content per 100 gm. of carcass tissue was slightly less than in the control group on the low fat stock diet. However, the tissue cholesterol content was definitely elevated when 3 per cent cholesterol was included in the diets, and this elevation appeared to be slightly greater in the rats on the corn oil diets both with and without added cholesterol. The opposite is indicated in the animals receiving lard plus choline, since a slightly lower tissue cholesterol content was found when the diet contained choline. A slight increase in the α-sterols of the fecal sterols from the rats on the more unsaturated fat diet is in agreement with a previous report[12] of this finding. A 12 per cent increase in another experiment also adds to this confirmation.

In Table III are summarized the percentages of the recovered sterols and metabolic end products of the sterols ingested during the four week period. The recovery of three to five times the amount of ingested sterol, when relatively small amounts were ingested, is evidence of considerable sterol synthesis in these animals although the presence of a larger proportion of plant sterols in the food may account for a sizable amount of the excretion.

The results suggest that only a limited amount of cholesterol is absorbed when 3 per cent is present in the diet. Less than 30 per cent of the ingested sterol was excreted as bile acids while approximately 50 per cent was recovered from the feces as sterols of which more than a half was still in the β-form. The recovery of slightly less fecal sterols and slightly more bile acids from the rats ingesting the corn oil plus cholesterol diet is in line with the suggestion[13] of an increased sterol absorption when fed with unsaturated fats. Again, considering per cent of total ingested sterol recovered from the carcass tissues instead of content per 100 gm. tissue, retention of sterol appeared to be a slightly greater in the carcasses of the rats on the corn oil and high cholesterol diet than when this fat was replaced with lard. The recovery of only approximately 80 per cent of the cholesterol ingested by these three groups of animals during the four week period suggests a sizable loss in some

TABLE III

Per Cent Recoveries of Metabolic End Products of Sterols Ingested During Four Week Period

Dietary Supplements	Ingested Sterols (gm.)	Fecal		Carcass Sterols (%)	Total Sterols Recovered (%)
		Sterols (%)	Bile Acids (%)		
Lard	0.69	203	286	13.0	502
Corn oil	1.35	197	113	12.6	323
Lard plus cholesterol	15.57	49	23	5.4	77
Corn oil plus cholesterol	12.16	46	28	6.9	80
Lard plus cholesterol and choline	14.52	51	25	5.0	81

TABLE IV

Activities Recovered One Week After Administration of Labeled Cholesterol to Rats on Diets Containing Lard, Corn Oil or No Added Lipid*

Dietary Supplements†	Per Cent of Total Activity Administered					
	Blood		Carcass	Liver	Total Fecal	Total Activities Recovered
	Relative Activities After 24 Hours	Recovered by Exsanguination				
None	730	1.87	49.4	8.7	41.8	101.8
Lard plus cholesterol	193	0.30	22.1	28.5	51.3	102.2
Corn oil plus cholesterol	170	0.26	8.5	25.9	52.3	87.0

* Cholesterol-4-C^{14} given after three weeks on diets and total activities were determined one week later.

† Supplemented diets contained 30 per cent of the fats, 2 per cent cholesterol and 0.4 per cent sodium taurocholate.

form which was not measured in this study.

The failure to obtain a more complete recovery of ingested sterol in these latter groups prompted a second experiment with labeled cholesterol in an attempt to more definitely establish results obtained in the balance study. Less cholesterol and a bile acid supplement were used in an attempt to obtain more complete absorption of the administered sterol. The results obtained suggest that the fate of a single ingestion of sterol after the rat had been on the diet for three weeks may not be that of the total ingested over an extended period.

The relative activities of the blood at eight and twenty-four hours after ingestion of the labeled cholesterol, as well as the per cent of total ingested activity recovered from the exsanguinated blood lipids, were again slightly lower in the animals receiving corn oil than in those receiving lard. Only the latter are included (Table IV). Approximately four and six times as much activity in the early and final blood samples, respectively, was obtained from the rats on the stock diet as from those on the high fat diets. Results obtained on recovered activity from the lipids of the carcasses in all groups were similar to those on blood except for a more marked decrease in activity of tissues after consuming the corn oil diet.

Similar amounts of activity were retained in the livers and, as in the first experiment, excreted in the feces of both groups on the high fat diets. Markedly less activity was present, however, in both the livers and feces of the rats on the stock diet. An incomplete recovery of the ingested activity was obtained

only from the group of animals on corn oil in this experiment.

COMMENTS

This balance study was undertaken to determine whether any major changes might occur in the disposition of all cholesterol ingested during a four week period when the diets contained fats generally believed to affect blood cholesterol levels. Such a balance study could only aid in the clarification of the mechanism involved in changes of blood cholesterol levels if the change was associated with a major alteration in one or more of the means of disposal of ingested cholesterol. The absolute amount of sterol included in the change of the serum level is very small. Hence it could involve only a limited amount of sterol unless the amount affected was turned over very rapidly. Most of the blood cholesterol is generally believed to have a slow rate of turnover.

Blood cholesterol levels higher than those of the controls were found after the ingestion of the more saturated fat, but similar levels were found after the ingestion of the unsaturated fat when the diet did not contain added cholesterol. Apparently, when excessive amounts are ingested, more sterol is absorbed by the rat than can be cared for by the usual means of disposal since the sterol level was elevated both in the blood and in the carcass tissues. The slight increase in carcass sterols associated with the lowered blood level after the ingestion of unsaturated fat could well be the means of such a blood change, since a reverse effect was indicated when the tissue content was somewhat decreased by giving choline with the saturated fat. Also, the absolute amounts involved in both cases could more than account for the change in the blood levels.

Variations in the total fecal losses of sterols and bile acids or of activities were insufficient to explain the changes in blood cholesterol levels, since the total fecal excretions were approximately the same in all groups receiving excess cholesterol, as determined by the methods used. If there was a difference in the products excreted, the slight decrease in the sterols excreted by the rats ingesting the unsaturated fat was balanced by a slight increase in the bile acids, making the total excretion almost the same in all three groups.

Perhaps the suggestion of Bergström,[14] that drastic changes in the dietary fat might cause great changes in intestinal flora and that much of the sterol may be transformed by microorganisms into products that are not determined by the methods used, may explain the recovery of only 80 per cent of the ingested sterol in the last three groups. However, the change in blood sterol levels cannot be explained on the basis of this unaccounted for fraction (20 per cent), since a similar amount was missing in all three groups of rats on the high cholesterol diets.

The results obtained after a single ingestion of labeled cholesterol are in agreement with those in the balance study with two exceptions. Considerably less of the labeled sterol was retained in the carcass tissues of the rats receiving the unsaturated fat. This might be explained by the slightly greater increase in saturation of the tissues with the sterol after the rats had been on corn oil and cholesterol for three weeks. This suggestion of relative saturation is supported by marked increases in activity found in the blood and carcasses as well as the decrease in the excretions of those animals which had not been on the high cholesterol diets. A more rapid utilization of the sterol in the latter case would be expected to be associated with a more rapid turnover in the liver and hence less final retention of activity in that tissue.

The lowered total recovery of activity after ingestion of the unsaturated fat might indicate a different disposition of some of the sterol, unaccounted for by the methods employed. However, the over-all results are in agreement with those of a previous report[12] that the total recovery of excreted C^{14}-labeled end products of cholesterol metabolism was consistently higher from rats fed corn oil and lard than from those fed fat-free diets. Again, this may possibly be explained by the higher saturation of tissues with the sterol after high cholesterol intakes.

It is of interest to note that the data could

perhaps be more readily interpreted differently from those of the past. The saturated fats appear to have a much greater effect in elevating the blood cholesterol level than the unsaturated fats have in lowering it. Without additions of cholesterol to the diet, lard intake was associated with a high level of sterol in the blood whereas the level after ingestion of the corn oil was the same as that of the control. The level after ingestion of corn oil with cholesterol was higher than with the fat alone but, as indicated by activity measurements in the second experiment, possibly no higher than it would have been if the sterol had been ingested in the stock diet without added fat. Again there was about twice the percentage increase in the level when the corn oil was replaced with lard. Is it not possible that most of the effect of the unsaturated fats in the diet is largely a removal of the elevating effect of the saturated fats by simple replacement?

This reasoning is supported teleologically by four reported findings. (1) Saturated fats are about twice as effective in elevating the blood sterol level as the more unsaturated fats are in lowering it.[15] (2) Decreased absorption of long chain saturated fats when ingested in excess may be a means of protecting the organism against this harmful effect. (3) Even the reported increase in the rate of mitochondrial oxidation of cholesterol after feeding a saturated rather than an unsaturated fat[16] may be an added mechanism for eliminating a part of the excess thereby ameliorating the undesirable effect of saturated fats. (4) Large intakes of highly unsaturated fats are required to effect a lowering of the blood cholesterol level, perhaps by replacing the saturated fats, although some lowering occurs when a low fat diet is ingested.[17] It seems possible, therefore, that the emphasis in the past may have been wrongly placed on the effectiveness of the unsaturated or essential fatty acids in lowering the serum cholesterol level rather than on the more marked elevating effect on the blood level of the saturated fatty acids.

SUMMARY

Rats were fed diets containing 30 per cent corn oil or lard with and without cholesterol and choline for four weeks. An attempt was made to learn more about the mechanism involved in changes in the blood cholesterol level associated with such diets by comparing the total amoung of sterol ingested with the recovered from the carcass tissues, fecal sterols and bile acids. Similar recoveries of activities were attempted in another group of animals given cholesterol-4-C^{14} after three weeks on similar diets.

Sterol levels were found to be higher in the blood and carcass tissues of all animals on diets containing excessive amounts of cholesterol, even though only a limited amount of it appeared to be absorbed. The blood sterol level was elevated when dietary lard replaced the corn oil, whereas the tissue content appeared to be slightly increased by the intake of the more unsaturated fat.

The amount of fecal sterols and bile acids or activity excreted by the rats on the corn oil and lard-containing diets was similar whereas in those on the low fat diet more activity was retained in the blood and carcasses and less was excreted.

Lack of major changes in the disposition of ingested sterol in rats on the high fat diets prevents any definitite conclusions as to their role in the mechanism involved in blood cholesterol changes. The mass of conflicting reports attests to the increasing belief that this problem cannot be resolved without a more basic approach, probably at the cellular or subcellular level. However, the data obtained in this investigation and in many others are quite in line with the suggestion that the major changes in the blood cholesterol level may be caused by the *ingested saturated* fat rather than the unsaturated fat.

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