Diet, Cholesterol Metabolism, and Atherosclerosis

K. K. CARROLL,[1] The Collip Medical Research Laboratory,
University of Western Ontario, London, Ontario, Canada

Abstract

Effects of diet on acetate incorporation into cholesterol and fatty acids in liver slices, and on the level of plasma cholesterol, were studied in rats and rabbits. Feeding fats and oils in a commercial diet stimulated acetate incorporation into rat-liver cholesterol much more than feeding them in a semisynthetic diet. This effect seemed to be specific for cholesterol since incorporation into fatty acids was not similarly affected. High levels of dietary casein inhibited acetate incorporation into both cholesterol and fatty acids. Rat-liver slices generally incorporated more acetate into cholesterol than rabbit-liver slices, but incorporation into fatty acids was often higher in the latter.

Rabbit plasma cholesterols were higher on butter diets than on corn oil diets. Further elevation of plasma cholesterol was observed when casein was added to the butter diet but not when it was added to the corn oil diet. Plasma cholesterols were elevated, and acetate incorporation into liver cholesterol and fatty acids was inhibited in suckling rats and rabbits whereas recently weaned animals gave results similar to those of adults. The inverse relationship between plasma cholesterol level and acetate incorporation into cholesterol may be attributable to feedback control of liver cholesterol biosynthesis. Other mechanisms which may account for the observed effects of dietary fats and protein on cholesterol metabolism, and the possible relevance of the findings to atherosclerosis, are discussed.

Introduction

ATTEMPTS TO PRODUCE ATHEROSCLEROSIS in experimental animals were unsuccessful until the early years of this century when Ignatowski and others showed that rabbits develop atherosclerotic lesions when fed on meat, milk, and eggs (1–3). At first it was thought that the high protein content of these diets was responsible for the effect, but later experiments by Anitschkow and Chalatow (3,4) and by Wacker and Hueck (5) showed that similar results could be produced by feeding cholesterol. The focus of attention then centered on cholesterol, and much of the subsequent work on experimental atherosclerosis has been carried out with diets containing added cholesterol (3,6–10). Although this has yielded much valuable information, the amounts of cholesterol have, in general, been much greater than would be present in natural diets of ether human beings or experimental animals (11), and such diets are therefore not satisfactory for exploring the metabolism of cholesterol under normal conditions. Further, it seems probable that the emphasis on dietary cholesterol has tended to obscure the effects of other dietary components which may play an important role in the etiology of atherosclerosis.

A significant development within the past 10 years has been the discovery that atherosclerotic lesions can be produced in rabbits by feeding diets containing little or no cholesterol (12–14). In general, semisynthetic diets appear to be more effective than stock diets in producing lesions under these conditions (15). The presence of fat in these diets is not essential, but the addition of saturated fat increases their potential for producing atherosclerosis (16).

These results demonstrate that atherosclerosis can occur in the absence of dietary cholesterol, but the level of serum cholesterol nevertheless appears to be a significant factor in its development (17). Hypercholesterolemia usually precedes the formation of atherosclerotic lesions in experimental animals (6,8), and this is also true of rabbits fed low-cholesterol, semisynthetic diets (14). Since these rabbits have little access to exogenous cholesterol, it appears that their serum cholesterol must be derived almost entirely from endogenous synthesis. It is well known that many body tissues can synthesize cholesterol from small molecule precursors (18,19), but serum cholesterol appears to come mainly from the liver and intestine (20). In species such as the rat and dog, the liver seems to be the main source (21–23), but in human beings a larger proportion apparently comes from other tissues (24,25).

There is considerable evidence that liver cholesterol synthesis is controlled by a feedback mechanism regulated by cholesterol and bile acids (26–29). Synthesis can be inhibited by administering cholesterol or bile acids and may be increased by cannulating either the bile duct (30) or the intestinal lymph ducts (31), thus interrupting the enterohepatic flow of cholesterol and bile acids. There is lack of agreement on the extent to which this feedback mechanism operates in man (32,33), but, in experimental animals at least, it may be an important controlling factor in the regulation of serum cholesterol levels.

Effects of dietary components other than cholesterol on liver cholesterol synthesis have not been studied extensively, but the role of dietary fat has been investigated in a number of different laboratories (34–40). In most cases it has been found that dietary fat stimulates incorporation of acetate into liver cholesterol, and unsaturated fat appears to be somewhat more effective than saturated fat. Studies in this laboratory gave results in agreement with these conclusions, and it was found, in addition, that fats fed in a commercial diet gave much greater stimulation than fats fed in a semisynthetic diet (41). These effects of diet seemed to be specific for acetate incorporation into cholesterol since corresponding changes were not seen for acetate incorporation into fatty acids, measured in the same experiments (Figure 1).

Other studies in this laboratory showed that acetate incorporation into both cholesterol and fatty acids was reduced in liver slices from suckling rats (42). As soon as the animals were weaned, incorporation quickly increased to normal adult levels, and acetate incorporation into fatty acids by livers from recently weaned rats was frequently much higher than in adult livers although there was considerable variation from animal to animal (Figure 2). It seemed likely that the inhibition observed in suckling rat liver was related to diet because there was a rapid change at weaning, also because fetal rat liver was found to

[1] Medical Research Associate of the Medical Research Council of Canada.

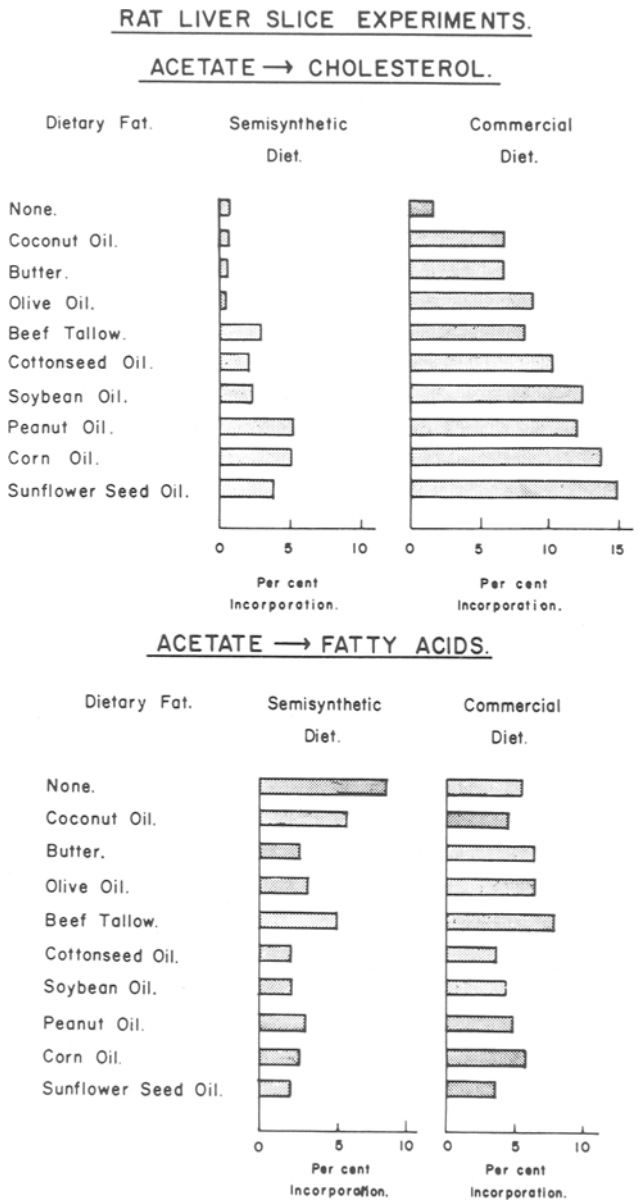

FIG. 1. Effects of diet on incorporation of acetate-1-C^{14} into cholesterol and fatty acids by rat-liver slices. The basic composition of the fat-free semisynthetic diet is given in Table II. The commercial diet was a Fox Breeder Starter Ration, which was extracted with ether to remove most of its endogenous fat. All fats and oils were incorporated into the two diets at a level of 15% by weight, and the diets were fed for approximately two weeks before the slice experiments were carried out. For further details see the original publication (41).

give good incorporation of acetate into cholesterol and fatty acids (42).

The in-vivo studies in which the labeled acetate was administered either orally or intraperitoneally also showed that liver cholesterol and fatty acids accumulated less radioactivity in suckling rats than in weaned rats (42). Similarly, in vivo experiments with rats on semisynthetic and commercial diets (unpublished) confirmed these results of in vitro studies. The stimulating effect of dietary fat on acetate incorporation into liver cholesterol has also been observed with in vivo as well as in vitro experiments (34,40).

The experiments with suckling rats provided evidence of an inverse correlation between acetate incorporation into liver cholesterol and the level of plasma cholesterol since plasma cholesterols were elevated in suckling rats and dropped to normal adult

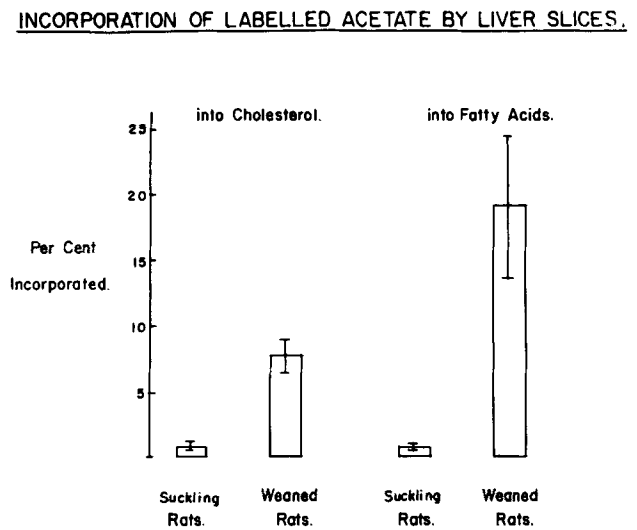

FIG. 2. Incorporation of acetate-1-C^{14} into cholesterol and fatty acids by liver slices from suckling and weaned rats. The animals were 13 to 17 and 30 to 35 days old respectively. There were six animals per group.

values at weaning (Figure 3). There is some indication of a similar inverse correlation from experiments involving the feeding of semisynthetic and commercial diets. In the studies, acetate incorporation into cholesterol was lower in rats fed semisynthetic diets (Figure 1), but Portman and others have found that rats fed semisynthetic diets have higher serum cholesterol levels than rats fed commercial diets (43). Also, as mentioned earlier, rabbits develop hypercholesterolemia and atherosclerotic lesions more readily on semisynthetic diets than on stock diets. These findings and other evidence of a relationship between liver cholesterol metabolism, serum cholesterol levels, and development of atherosclerotic lesions (26,44) prompted further investigations of the influence of diet on acetate incorporation into liver cholesterol.

Earlier studies had shown that incorporation was markedly influenced by the presence of fat in the diet and the type of fat which was fed (Figure 1). However it seemed that the nonlipid portion of the diet must also have a significant role since the stimulation obtained by adding fats to a commercial diet was always much greater than that obtained by adding them to a semisynthetic diet.[1] Therefore the main objective of subsequent experiments was the identification of nonlipid components of the diets which might

[1] In these experiments, most of the endogenous fat was first extracted from the commercial diet with ether, and experiments indicated that the small amount of lipid remaining was probably not responsible for the observed differences between commercial and semisynthetic diets.

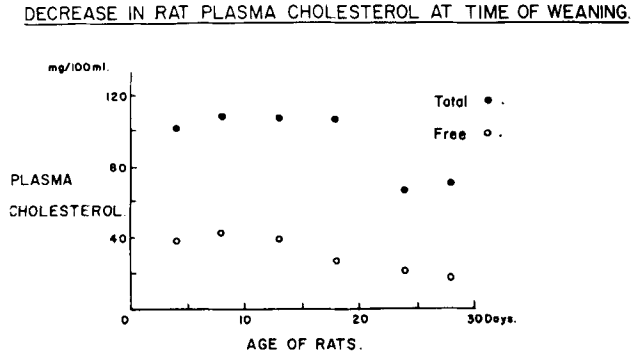

FIG. 3. Free and total plasma cholesterol levels in suckling and weaned rats. The animals were weaned at 20 to 21 days of age.

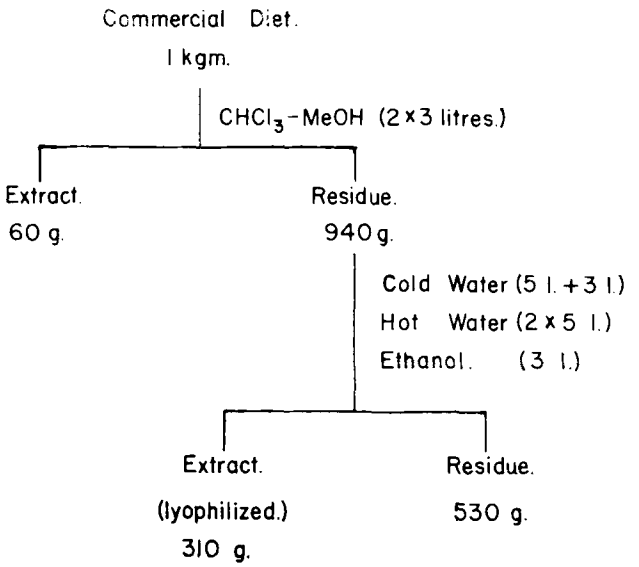

FIG. 4. Fractionation of rat commercial diet.

account for this difference. In addition, some experiments were carried out with rabbits as well as rats in order to compare effects of diet on cholesterol metabolism in two species which differ greatly in their susceptibility to atherosclerosis.

The problem of identifying nonlipid components responsible for differences between commercial and semisynthetic diets was approached in several different ways. In one set of experiments the nonlipid portion of the commercial diet was separated into water-soluble and water-insoluble fractions, and these were mixed with a semisynthetic diet and were fed to rats to test for stimulatory effects on acetate incorporation into cholesterol in the liver-slice system used in earlier experiments (41,45). In other studies the composition of the semisynthetic diet was varied. Components of the semisynthetic diet were also mixed with the commercial diet to determine whether they had any inhibitory effect on incorporation. In each of these studies, fat (usually butter, 15% by weight) was added in most cases to the experimental diets because the presence of fat accentuated the difference between results obtained with commercial and semisynthetic diets (Figure 1).

Experimental Procedure

Fractionation of Commercial Diet

Fractionation of the rat commercial diet (Master Fox Breeder Starter Ration, Maple Leaf Mills Ltd., Toronto, Ontario) was carried out according to the scheme shown in Figure 4. Chloroform-methanol (3:1) was used for the first two extractions. The residue was then extracted twice with water at room temperature, twice with water at near boiling temperatures, and once with ethanol at room temperature. The residues were separated from the extracts by filtration by using coarse filter paper for the chloroform-methanol extract and filter paper lined with a layer of cheesecloth for the aqueous extracts. The latter were more difficult to filter, and some losses inevitably occurred in scraping the residue from the filter paper and cheesecloth. This probably accounts for most of the loss in weight (Figure 4). The final extraction with ethanol made it easier to air-dry the insoluble residue. The combined aqueous-ethanolic extract was concentrated in vacuo before lyophilization.

The results of feeding the aqueous-ethanol extract and the residue, mixed in varying proportions with fat-free semisynthetic diet, are shown in Table I.

In Experiments 1 and 2, extract and residue were fed in approximate proportion to their amount in the commercial diet whereas in Experiments 3 and 4 they were fed on an equal-weight basis. Acetate incorporation into cholesterol was enhanced by both fractions of commercial diet in all cases, and the amount of acetate incorporated seemed to increase as the proportion of either fraction in the diet increased. Because of the variability in the results it is difficult to say whether one fraction was more effective than the other.

These experiments failed to localize the stimulatory effect of the commercial diet, and this failure raised the possibility that the stimulation was only apparent and was, in fact, attributable merely to the dilution of inhibitory factors in the semisynthetic diet. To explore this, further experiments were carried out to determine whether any components of the semisynthetic diet could be demonstrated to have a specific inhibitory effect on acetate incorporation into cholesterol.

Effect of Different Components of Semisynthetic Diets

Semisynthetic diets containing 15% butter were used for these experiments, and the composition of the basic control-diet is given in Table II.

Substitution of other proteins for the casein in this diet or of other carbohydrates for the glucose did not seem to produce any significant change in incorporation (Table III).

Other alterations, such as the use of salt mixtures

TABLE I

Acetate Incorporation into Cholesterol by Rat Liver Slices
Effect of Feeding Commercial Diet Fractions Mixed with Semisynthetic Diet

Expt.	Diet[a]	No. of rats	Rat body weight (g)	% of counts from acetate recovered in cholesterol
1.	Semisynthetic (control)	3	124	1.5(0.6— 2.4)[b]
	Extract (34) + semisynthetic (66)	3	104	6.2(4.4— 9.6)
	Residue (72) + semisynthetic (28)	3	155	7.7(5.0—10.6)
2.	Semisynthetic (control)	3	158	1.1(0.8— 1.4)
	Extract (44) + semisynthetic (56)	3	122	3.1(1.5— 5.8)
	Residue (75) + semisynthetic (25)	3	161	4.6(2.1— 6.8)
3.	Semisynthetic (control)	3	167	1.6(1.0— 2.1)
	Extract (40) + semisynthetic (60)	4	136	5.2(1.3—10.3)
	Residue (40) + semisynthetic (60)	5	168	3.0(1.9— 5.5)
4.	Extract (20) + semisynthetic (80)	6	144	2.3(1.4— 3.6)
	Residue (20) + semisynthetic (80)	5	155	2.3(1.7— 3.2)

[a] Commercial diet extract or residue was mixed with fat-free semisynthetic diet (Table II) in proportions as indicated by the figures in brackets. Then 15% by weight of butter was added to each of the diets, including the semisynthetic control. Diets were fed for two-week periods as in earlier experiments.

[b] Average values and extremes are given.

TABLE II

Composition of Semisynthetic Diets

	Fat-free	Fat-containing
Casein	18 g	22 g
Dextrose	72 g	52 g
Fat	....	15 g
Salt mixture (Phillips-Hart)	4 g	5 g
Celluflour	5 g	5 g
Vitamin-mixture[a]	1.5 ml	1.5 ml

[a] Prepared by dissolving 50 mg of thiamine hydrochloride, 50 mg of pyridoxine hydrochloride, 2 mg of biotin, 10 g of choline chloride, and 1 g of inositol in 30 ml of water, then adding 100 mg of riboflavin, 500 mg of nicotinic acid, 590 mg of calcium pantothenate, and 5 mg of folic acid to 50 ml of ethyl alcohol. The two solutions were then mixed and made to 100 ml with water.

Sources of materials: casein (purified high nitrogen), salt mixture and vitamins, Nutritional Biochemicals, Cleveland, O.; dextrose, Ingram and Bell Ltd., Toronto, Ontario; Celluflour, Chicago Dietetic Supply House Inc., Chicago, Ill.

of different composition, omission of choline and inositol, reduction in the level of all water-soluble vitamins, or addition of extra Celluflour all failed to affect acetate incorporation into cholesterol. The only experiments which gave suggestive results were those in which the level of casein in the diet was varied at the expense of the dextrose. When casein was fed as 40% of the diet, incorporation was uniformly low whereas, when it was fed as only 10% of the diet, incorporation was higher than for any other semisynthetic diet tested although still considerably below that observed for rats on commercial diet.

The possible significance of this last set of results was examined further by mixing either casein or dextrose with commercial diet, adding 15% by weight of either butter or corn oil to the mixtures, and feeding these diets to rats. The results showed that the addition of casein depressed acetate incorporation into cholesterol when either the butter or corn oil-containing diet was fed whereas addition of dextrose had no such effect (Table IV).

Acetate incorporation into fatty acids was also measured in these experiments. Substitution of various components of the semisynthetic diet seemed to have little effect except in the case of substitution of sucrose for glucose where incorporation was greater (Table III). Omission of choline and inositol from the diet also seemed to stimulate incorporation, but, because of the variability of the results, these findings should be verified with larger groups of animals. The effects of casein on acetate incorporation into fatty acids paralleled to some extent its effects on acetate incorporation into cholesterol (Tables III and IV).

Effects of Diet on Cholesterol Metabolism in Rabbits

The rat is especially resistant to experimental atherosclerosis, and rat serum cholesterols are not easily altered. It therefore seemed of interest to repeat some of the experiments in rabbits, which are much more susceptible to atherosclerosis and to alteration of serum cholesterol levels.

Studies with liver slices from rabbits fed various diets showed that acetate incorporation into cholesterol was significantly lower than in similar experiments with rats (Tables IV and V). As in the rat experiments, the addition of casein to commercial diet depressed incorporation somewhat, but the differences were not great. Acetate incorporation into fatty acids was generally higher in rabbit-liver slices than in rat-liver slices although the results were extremely variable in the rabbit experiments.

Measurement of plasma cholesterols showed interesting differences between groups of rabbits fed different butter-containing diets (Table V). When butter was fed in a semisynthetic diet, the rabbits had a higher average plasma cholesterol level than when it was fed in a commercial diet. However, when the commercial diet was mixed with casein, the average cholesterol level was even higher than that obtained with the semisynthetic diet. But the lowest average plasma cholesterol for a butter-containing diet was found in rabbits which were fed commercial diet mixed with dextrose. The livers of this last group had a somewhat fatty appearance, suggesting a defect in transport of fat from liver to plasma.

Rabbits fed diets containing corn oil had lower plasma cholesterols than most of those in the butter-fed groups, in agreement with results from other laboratories (14). Addition of either casein or dextrose to the commercial diet seemed to make little difference when corn oil was used as the dietary fat.

Experiments were also carried out with suckling and recently weaned rabbits, and the results (Table VI) were analogous to those obtained earlier with rats. Liver slices from suckling rabbits gave low incorporation of acetate into cholesterol, and the plasma cholesterol levels were considerably higher than those

TABLE III

Acetate Incorporation into Cholesterol and Fatty Acids by Rat-Liver Slices
Effect of Variations in Nonlipid Components of Semisynthetic Diet

Type of diet[a]	No. of animals	Rat body weight (g)	% of counts from acetate recovered in cholesterol	% of counts from acetate recovered in fatty acids
Semisynthetic diet (control)	9	149	1.2(0.6—2.2)[b]	3.6(1.3—10.1)[b]
Lactalbumin replacing casein	3	155	2.2(1.4—3.5)	2.5(1.7— 3.9)
Wheat gluten replacing casein	3	118	1.8(0.7—2.7)	3.2(1.8— 5.4)
Soya protein replacing casein	3	111	1.5(0.6—2.2)	3.1(2.9— 3.6)
Sucrose replacing dextrose	3	139	1.5(0.2—2.4)	9.9(4.0—14.5)
Wheat starch replacing dextrose	3	149	0.6(0.3—0.8)	2.2(1.9— 2.7)
Corn starch replacing dextrose	3	162	1.1(0.3—2.3)	2.8(1.9— 4.2)
MD-185 salt mix replacing PH salt mix	3	156	1.0(0.2—1.4)	3.2(1.1— 5.7)
Calcium-free salt mix replacing PH salt mix	3	139	0.2(0.1—0.3)	2.2(1.2— 2.8)
HMW salt mix replacing PH salt mix	3	173	1.5(1.1—1.8)	3.5(3.2— 4.0)
Choline and inositol omitted	3	158	1.0(0.7—1.5)	6.2(2.9—11.5)
Water-soluble vitamin supplement reduced to ⅓ of normal	3	166	1.6(1.0—2.4)	3.1(2.7— 3.4)
Additional 20% Celluflour added	4	162	0.8(0.4—1.1)	2.5(1.8— 3.2)
40% Casein	6	171	0.3(0.1—0.5)	2.0(1.4— 2.8)
30% Casein	3	150	1.6(0.6—3.6)	2.9(2.3— 3.4)
20% Casein[c]	9	149	1.2(0.6—2.2)	3.6(1.3—10.1)
10% Casein	6	107	2.9(1.3—7.2)	4.8(2.9— 8.7)

[a] Diets were fed for two-week periods. See Table II for composition of basic semisynthetic diet. Replacement proteins, carbohydrates, and salt mixtures were obtained from Nutritional Biochemicals, Cleveland, O. except for sucrose and corn starch, which were purchased locally. MD-185 salt mix, formulated per McCollum and Davis, HMW salt mix, formulated by Hubbell, Mendel, and Wakeman. Compositions and original references given in a Diets Manual available from Nutritional Biochemicals.

[b] Average values and extremes are given.

[c] The same as the control diet above.

TABLE IV
Acetate Incorporation into Cholesterol and Fatty Acids by Rat Liver Slices
Effect of Adding Casein or Dextrose to Commercial Diet

Type of diet[a]	No. of animals	Rat body weight (g)	% of counts from acetate recovered in cholesterol	% of counts from acetate recovered in fatty acids
Diets containing 15% butter				
Ether-extracted commercial diet	4	153	10.7 ± 3.0[b]	6.8 ± 1.2[b]
25% casein added	4	177	4.7 ± 0.5	3.7 ± 0.6
25% dextrose added	4	151	8.8 ± 1.1	9.6 ± 1.1
Diets containing 15% corn oil				
Ether-extracted commercial diet	3	171	11.5 ± 4.2	3.7 ± 1.4
25% casein added	4	161	4.5 ± 1.0	2.4 ± 0.4
25% dextrose added	4	148	11.5 ± 1.1	3.6 ± 0.3

[a] Diets were fed for two-week periods.
[b] Average value ± standard error of the mean.

observed in suckling rats (Figure 3). In recently weaned rabbits the amount of acetate incorporated into cholesterol by liver slices was much greater, and the plasma cholesterol had decreased to a level similar to that of normal adult rabbits.

Discussion

The experiments which have been described represent an attempt to identify dietary components which affect liver cholesterol synthesis in experimental animals. In some experiments plasma cholesterol levels were also measured to investigate possible relationships between plasma levels and liver synthesis, and studies were carried out in both rats and rabbits to obtain comparative data for species which differ greatly in susceptibility to atherosclerosis.

In studies in this laboratory, as well as in those of other workers (34–37,39,40), dietary fat was found to stimulate acetate incorporation into cholesterol by rat liver preparations. However the degree of stimulation appeared to depend on the type of fat and to an even greater extent on the kind of diet in which the fat was fed. Fats were much more effective when fed in a commercial diet than when fed in a semisynthetic diet (Figure 1). Attempts to identify nonfatty components of these diets which might account for the difference were relatively unsuccessful, and the only positive results were obtained by altering the protein content. Incorporation seemed to vary inversely with the level of casein in the semisynthetic diet (Table III), and addition of casein to commercial diet decreased incorporation in both rats and rabbits (Tables IV and V).

Measurement of acetate incorporation into both cholesterol and fatty acids in the same experiments indicated that the effects of dietary fat were specific for cholesterol synthesis (Figure 1). This suggested that such factors as rate of penetration of acetate into the tissue slices, rate of activation of acetate, and size of the acetyl coenzyme A pool were not responsible for the observed differences. The results with semisynthetic diets gave some indication of a reciprocal relationship between acetate incorporation into cholesterol and its incorporation into fatty acids, which might indicate that the two pathways were competing for acetate. However this seems less likely when the results with commercial diets are also considered. Further, in the experiments in which the protein content of the diet was varied (Tables III-V), as well as in the experiments with suckling and recently weaned animals (Figure 2, Table VI), incorporation of acetate into fatty acids varied in the same direction as incorporation into cholesterol rather than inversely.

There is a good deal of evidence that cholesterol synthesis in rat liver is subject to feedback control (26–29), and this may be a factor in the responses to dietary ingredients. The finding that dietary effects were mediated at a point between acetate and mevalonate in the cholesterol biosynthetic pathway (41,42) is compatible with this possibility since one of the steps in that sequence, the conversion of hydroxymethylglutaryl coenzyme A to mevalonic acid, is generally considered to be the physiological site of feedback control (29).

In experiments with semisynthetic and commercial diets it seemed unlikely that dietary cholesterol would be a significant factor in feedback control since the lowest incorporation rates were obtained with semisynthetic diets, which contained no added cholesterol other than that present in some of the fats used (e.g., butter). It is however possible that other dietary components may affect the transport, degradation, and excretion of cholesterol in such a way as to alter the size or distribution of the cholesterol pool and so affect liver cholesterol synthesis in this way via feedback control.

Studies in other laboratories have given some indication that catabolism and excretion of cholesterol is increased by dietary fat (39,46). There are also reports that rats and rabbits fed commercial diets excrete more fecal bile acids than those fed semisynthetic diets (43,47). Loss of cholesterol in this way could stimulate liver cholesterol synthesis. Another possibility is that dietary components have a primary effect on liver lipoprotein synthesis, which in turn could cause an alteration in cholesterol synthesis since cholesterol is a major component of the lipoproteins synthesized by the liver (48). Experimental evidence indicates that about one-third of absorbed dietary triglyceride goes more or less directly to the liver, where part of it is rapidly incorporated

TABLE V
Effects of Diet on Cholesterol Metabolism in Rabbits

Diet	No. of rabbits	Body weight (kg)	Acetate incorporation by liver slices[a]		Plasma cholesterol (mg/100 ml)	
			% incorp. into cholesterol	% incorp. into fatty acids	Free	Total
Diets containing 15% butter						
Semisynthetic	4	1.78	1.6 ± 0.9[b]	21.7 ± 8.3[b]	48 ± 5[b]	148 ± 13[b]
Rabbit pellets[c]	9	1.40	2.1 ± 0.6	8.4 ± 3.7	41 ± 3	112 ± 12
+ 25% casein	6	1.65	0.5 ± 0.2	8.6 ± 3.9	68 ± 11	170 ± 11
+ 25% dextrose	6	1.32	3.1 ± 1.2	20.6 ± 6.4	52 ± 10	85 ± 6
Diets containing 15% corn oil						
Rabbit pellets[c]	6	1.70	2.5 ± 0.8	8.7 ± 1.3	34 ± 4	78 ± 14
+ 25% casein	6	1.76	1.0 ± 0.3	4.4 ± 1.0	33 ± 7	76 ± 7
+ 25% dextrose	6	1.58	2.1 ± 0.8	6.9 ± 2.7	61 ± 16	88 ± 20

[a] Diets were fed for two-week periods and experiments carried out in the same way as for rat-liver slices.
[b] Average value ± standard error of the mean.
[c] Master Rabbit Pellets, Maple Leaf Mills, Toronto, Ontario. These were ground and fed in powdered form in all diets.

TABLE VI

Cholesterol Metabolism in Suckling and Weaned Rabbits

Age (days)	Body weight[a] (g)	Acetate incorporation by liver slices		Plasma cholesterol (mg/100 ml)	
		% incorp. into cholesterol	% incorp. into fatty acids	Free	Total
		Suckling rabbits			
16	163	0.4 ± 0.3[b]	3.6 ± 1.8[b]	101 ± 16[b]	311 ± 33[b]
21	191	0.1 ± 0.03	0.4 ± 0.1	141 ± 3	522 ± 11
		Weaned rabbits			
36	822	7.9 ± 2.0	8.5 ± 3.9	21 ± 3	58 ± 5

[a] Four rabbits in each group.
[b] Average value ± standard error of the mean.

into newly-formed lipoproteins and again released into the blood stream (49,50). Increased amounts of dietary fat could thus stimulate liver lipoprotein formation and lead to increased cholesterol synthesis. The major portion of the cholesterol in plasma lipoproteins is in esterified form, and it is of interest that Avigan and Steinberg (34) found that dietary fat caused an increase in the amount of esterified cholesterol in rat liver. Corn oil had a greater effect than coconut oil. Dietary protein might also affect cholesterol metabolism indirectly as a result of effects on lipoprotein synthesis, but there are few data available regarding the effects of dietary components on liver lipoprotein synthesis (48).

If liver cholesterol synthesis is controlled, at least in part, by the rate of entry of cholesterol into the liver, one might expect to find an inverse relationship between acetate incorporation into liver cholesterol and the level of plasma cholesterol. It is unlikely that such a relationship would hold in all cases because the level of plasma cholesterol is not necessarily an index of the rate of entry of cholesterol into the liver or of the amounts which arrive at the site of feedback control. However, in some of the experiments, there was evidence of an inverse relationship, particularly in the case of suckling animals (Figures 2 and 3, Table VI). The elevated plasma cholesterols and low acetate incorporation in these animals may be largely owing to dietary cholesterol derived from the milk (51), but it is possible that other components of milk are also involved. In this connection it may be noted that the addition of casein to a butter-containing diet gave a significant elevation of the average plasma cholesterol in rabbits whereas it had no effect when added to a corn oil-containing diet (Table VI). This raises the possibility that dietary protein may be a factor in the absorption of cholesterol from the intestine.

It is obvious from these and other experiments dealing with cholesterol metabolism that interactions between dietary components can have an important bearing on the results obtained. This is perhaps one of the main reasons why it has been so difficult to assess the role of different dietary components in the etiology of atherosclerosis. The widespread use of relatively high levels of dietary cholesterol as a means of producing atherosclerosis in experimental animals may have also helped to complicate the picture.

The discovery that atherosclerosis can be produced in rabbits on low-cholesterol diets offers a more satisfying experimental model for studying effects of other dietary components on cholesterol metabolism in relation to atherosclerosis. Although great caution must be exercised in extrapolating results of animal experimentation to the situation in man, the responses of serum cholesterol levels in rabbits fed low-cholesterol diets to changes in dietary fat bear at least some resemblance to those observed in human beings.

Experiments in human beings have demonstrated that the level of serum cholesterol is markedly reduced by feeding low-fat diets (11,52,53), and the pioneering studies of Kinsell et al. (54,55) and Groen et al. (56), showing that serum cholesterol levels are affected by type as well as quantity of dietary fat, have been confirmed in many laboratories (57–59). It is generally agreed that fats containing substantial amounts of polyunsaturated fatty acids depress serum cholesterol levels in most human subjects whereas fats containing predominately saturated fatty acids have the opposite effect. Similarly, in rabbits on low cholesterol diets, low levels of dietary fat are associated with low serum cholesterol levels, and the highest serum cholesterol levels are obtained with diets containing saturated fats (13,14,16,60,61).

The mechanisms by which these effects are mediated in human beings are being actively studied in a number of different laboratories. Wood, Shioda, and Kinsell (62) have recently reported that the total fecal loss of steroids is lowest on a fat-free diet, intermediate on a diet containing saturated fat, and highest on a diet containing polyunsaturated fat. This agrees with earlier findings by other workers, but studies in several laboratories have provided strong evidence that increased fecal excretion of sterols and bile acids alone cannot account for the lowering of serum cholesterol level when unsaturated fats are substituted for saturated fats in the diet (63–65). Bieberdorf and Wilson (61) reached a similar conclusion in their studies with rabbits on cholesterol-free diets, in which a lowering of serum cholesterol was achieved by substituting corn oil for hydrogenated coconut oil. They provided some evidence that the drop in serum cholesterol level was caused by a redistribution of cholesterol between the serum and other body tissues such as muscle, and a similar suggestion has been made to account for the results obtained in human studies (63).

Dietary fat tends to raise the level of serum cholesterol in rats on low-cholesterol diets (34,39,66–71), and rats fed semisynthetic diets have somewhat higher serum cholesterol levels than those fed commercial diets (43,69), but the differences are not as great as those observed in rabbits. Yet dietary alterations of this sort appear to have a greater effect on acetate incorporation into liver cholesterol in rats (Figure 1) than they do in rabbits (Table V). Incorporation is lower in rabbits in most cases, in agreement with the findings of Cox et al. (25), who commented that there appears to be a general correlation between the rate of hepatic cholesterol synthesis and resistance to the induction of hypercholesterolemia by feeding cholesterol. A high rate of hepatic synthesis may allow

more opportunity to compensate for dietary cholesterol intake by feedback inhibition. The rate of hepatic cholesterol synthesis and the importance of feedback control in human beings as a mechanism of compensating for dietary cholesterol intake is still subject to controversy (32,33).

Effects of dietary protein on blood cholesterol levels and production of atherosclerosis in experimental animals have been studied in a number of different laboratories with varying results (6,72,73). In many cases the interpretation is complicated by the use of diets containing 1% to 5% of cholesterol, but there is some evidence that casein can produce elevated blood cholesterol levels and atherosclerotic lesions in rabbits on a diet essentially free of cholesterol (74). As noted earlier, experiments showed that casein promoted hypercholesterolemia in rabbits fed a butter-containing diet and tended to inhibit acetate incorporation into cholesterol in both rat- and rabbit-liver slices. In attempting to determine why rabbits develop hypercholesterolemia and atherosclerotic lesions on semisynthetic diets, Howard et al. (16,75) found that replacing casein by hexane-extracted soybean meal in a semisynthetic diet prevented the effects. They postulated an active factor in the soybean meal, but attempts to isolate such a factor were unsuccessful. Kritchevsky and Tepper (76) have obtained additional evidence that the nonfatty portion of the diet is responsible for the difference in atherogenic potential between semisynthetic and commercial diets.

There is also lack of agreement in regard to the effects of dietary protein on serum cholesterol levels in human beings. Several groups of workers found that dietary protein had no effect (77,78), but Olson et al. (79) observed that human subjects transferred from a diet containing 100 g of protein derived largely from animal sources to one containing 25 g of protein derived from vegetable sources showed a significant drop in serum cholesterol levels. Further experiments suggested that this may be related to levels of nonessential amino acids in the diet (80). Evidence from some epidemiological studies suggests that coronary mortality can be better correlated statistically with animal protein intake than with fat intake (81,82). Other evidence of a correlation between protein intake and serum cholesterol levels has been reviewed by Olson and Vester (83).

A number of recent publications have suggested that dietary carbohydrate, particularly simple sugars, may be an important factor in the etiology of atherosclerosis (84–87). Studies with rat- and rabbit-liver slices gave no indication that dietary carbohydrate influenced acetate incorporation into cholesterol (Tables III and IV), but there was suggestive evidence that substitution of sucrose for dextrose in a semisynthetic diet increased acetate incorporation into fatty acids (Table III). The addition of dextrose to a butter-containing diet caused a definite lowering of the plasma cholesterol level (Table IV), but this effect may have been owing to a reduction in the protein level of the diet rather than to an increase in carbohydrate content.

The increase in serum cholesterol level which occurs in both experimental animals and human beings soon after birth while they are subsisting largely, if not entirely, on a milk diet is deserving of further consideration. In rabbits this rise has been attributed to the cholesterol furnished by the milk (51), but recent studies by Harris et al. (88) provide evidence that in suckling rats the hypercholesterolemia is attributable to the high fat content of the milk rather than its cholesterol content. Other components of milk, such as casein and lactose, may also play a role since there is evidence that each can elevate serum cholesterol levels (74,89). The possibility that these various components are more effective in combination than when fed singly must also be considered.

At the time of weaning the blood cholesterol level drops promptly in rats and rabbits (Figure 3, Table VI), but in human beings it remains elevated (90,91). This difference may be caused, at least in part, by the continued use of substantial amounts of milk and milk products in human diets post-weaning. Studies with human infants have shown that the rise in serum cholesterol levels after birth can be largely prevented by using diet formulae in which the milk is partially or wholly replaced by such ingredients as corn oil and soybean products (92,93).

The possible relationship between serum cholesterol levels and the development of atherosclerotic lesions in young animals, as in adults, is open to question. However Ssolowjew (94) observed fatty streaks in the aortas of suckling rabbits which disappeared after weaning, and these results were confirmed and extended by Bragdon (95). Furthermore the relatively early appearance of fatty streaks in human aortas (96) may be related to milk and dietary fat intake and the sustained increase in serum cholesterol level in the early years of growth. There is also some evidence that the use of Sippy diets and others high in milk content for the treatment of peptic ulcer in adults is associated with a high incidence of myocardial infarction (97). Because of these and other findings, milk and milk products are widely considered to be atherogenic foods (98), but their possible deleterious effects in this regard must be balanced against their acknowledged high nutritional value (99).

Summary and Conclusions

Atherosclerosis is commonly produced in experimental animals by dietary means, and there is epidemiological evidence to suggest that diet plays a significant role in human atherosclerosis as well. Cholesterol is also linked to atherosclerosis by its presence in atherosclerotic plaques, by its ability to produce atherosclerotic lesions when fed to experimental animals, and by evidence of a positive correlation between blood plasma cholesterol levels and the development of atherosclerosis in experimental animals and in man.

Plasma cholesterol is derived both from the diet and from endogenous synthesis in body tissues, but the mechanisms which determine plasma cholesterol levels are poorly understood and experimental work on the problem is complicated by species differences. The liver appears to be a major source of plasma cholesterol, at least in some species, and the present studies were carried out in an attempt to understand how liver cholesterol synthesis is affected by diet and how this in turn may correlate with plasma cholesterol levels. Rats and rabbits, which differ greatly in susceptibility to atherosclerosis, were used for the experiments, with the hope of obtaining a better understanding of the reasons for this species difference.

The results showed that liver cholesterol synthesis, as measured by acetate incorporation into cholesterol, was depressed in animals fed semisynthetic diets in comparison with animals fed commercial diets. Di-

etary fat in most cases stimulated incorporation whereas dietary protein tended to have an inhibitory effect. Suckling animals showed low rates of liver cholesterol synthesis, which is associated with elevated plasma cholesterol levels. Animals on semisynthetic diets also tended to have higher blood cholesterol levels than animals on commercial diets. This negative correlation between liver cholesterol synthesis and plasma cholesterol levels can perhaps be explained on the basis of feedback control of liver synthesis. The level of plasma cholesterol was more readily altered in rabbits than in rats, but acetate incorporation into liver cholesterol was generally higher in rats and showed greater differences in response to diet. This increased ability to vary the rate of liver cholesterol synthesis may help to stabilize plasma cholesterol levels in the rat.

These results are of interest in relation to reports from other laboratories that atherosclerotic lesions develop in suckling rabbits and in rabbits fed semisynthetic diets but not in rabbits fed stock diets. In these studies, as well as in this laboratory's work, the diets contained no added cholesterol. For investigating effects of dietary components on cholesterol metabolism in relation to atherosclerosis, such diets appear to be preferable to the high-cholesterol diets which have been widely used for producing atherosclerosis in experimental animals.

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