Cholesterol turnover, synthesis, and retention in hypercholesterolemic growing swine

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Abstract Studies of the interaction of cholesterol absorption, excretion, synthesis, and retention were carried out with growing swine through the application of sterol and isotopic balance procedures to a non-steady state situation. The animals were fed either a low-fat commercial mash diet (MA), a commercial mash diet plus 2.4 g/day of crystalline cholesterol (MAC), or a high-fat milk diet containing 2.4 g/day cholesterol (MKC). [^{14}C]Cholesterol and β-[^{3}H]sitosterol were used to measure fecal neutral steroid losses. Final plasma cholesterol levels averaged 91, 85, and 195 mg/100 ml for MA, MAC, and MKC animals, respectively. Total carcass dissection followed by cholesterol measurement revealed that accumulation of cholesterol in most body tissues was directly related to increase in body size in the rapidly growing swine. The only notable exceptions were plasma, liver, and bone with marrow of the MKC group, where cholesterol concentrations were significantly elevated. Absorption of cholesterol was significantly greater in animals fed MKC than MAC-fed swine (726 mg/day vs. 142 mg/day), whereas synthesis of cholesterol was significantly greater in MAC animals than in MKC animals (863 mg/day vs. 112 mg/day). Excretion of neutral and acid steroids into the intestine was not significantly different between MAC and MKC groups, but both classes of steroids were more efficiently reabsorbed in MKC animals. The overall differences between swine fed mash–cholesterol and those fed milk–cholesterol diets appear to result from more efficient absorption of both neutral and acid steroids in the milk–cholesterol group only partially compensated for by decreased cholesterol synthesis.

Supplementary key words cholesterol absorption · fecal steroids · sterol balance · non-steady state · sterol degradation · tissue cholesterol pools · feedback inhibition of cholesterol synthesis · β-sitosterol

THE PRESENT STUDY was planned to measure cholesterol turnover, synthesis, and retention in swine fed high- and low-cholesterol diets. Methods for studying various aspects of cholesterol metabolism in man have been developed in the past decade (1–12). Several of these studies, furthermore, have dealt with overall sterol balance (4–7, 9, 10, 12). In such studies, however, the retention of cholesterol in man cannot be readily determined. This difficulty limits any conclusions regarding overall shift in body cholesterol pools. In order to circumvent the problem, present studies usually employ steady state conditions, which restrict the type of experimental situation that can be explored. In addition, one cannot always be certain that the required steady state condition has been achieved. Restrictions on cholesterol balance studies in man have led us to believe that studies in depth with a suitable experimental animal could provide useful information on mechanisms of steroid balance that cannot be obtained at the present time for man. Swine were chosen because (*1*) preliminary studies suggested that they are more like the human in their response to high-fat, high-cholesterol diets than are most other experimental animals including subhuman primates (13); (*2*) they eat virtually all of any reasonable quantity or quality of diet that is given to them; and (*3*) they develop atherosclerotic lesions that are in many ways similar to those in man (13). Young growing swine were chosen instead of mature swine because this period in life corresponds to the period (childhood) in which man begins to develop atherosclerosis and because we wanted to explore the feasibility of performing studies in animals that were not in a steady state.

In the current study, swine were fed a high-fat milk–cholesterol diet (MKC), a low-fat commercial mash–cholesterol diet (MAC), or a low-fat commercial mash diet without added cholesterol (MA) for periods of 22 or 31 days. Isotopic cholesterol was administered intravenously at the start of feeding so that isotope dilution techniques could be utilized. Fecal excretion of neutral

and acid steroids was measured chromatographically (5, 6), and daily fecal output was measured by use of a chromium oxide marker (14). Losses of fecal neutral steroids, possibly through bacterial transformation, were corrected for by use of isotope recovery techniques rather than recoveries of β-sitosterol (15). The validity of this approach for studies in swine was checked and is discussed in this report. Total carcass dissection at the termination of the experiment made it possible to measure the total body cholesterol content of each animal and thereby calculate total body cholesterol concentrations. We found that we could then estimate with reasonable accuracy the daily rate of cholesterol retention within each animal. Finally, a mathematical approach was utilized whereby calculation of daily rates of synthesis, endogenous excretion into the intestine, and dietary plus total absorption of cholesterol from the intestine was possible. This approach is an adaptation of well-known theoretical formulations (16, 17) to our specific system.

METHODS

Experimental animals

12 male Yorkshire swine approximately 8 wk old, having an average weight of 11.5 kg, were randomly placed in three groups of four animals each and were caged individually in slat-bottomed cages. For 1 wk prior to the experiment all animals were fed 630 g of a commercial mash (MA) diet daily (Table 1); 100 mg of chromium oxide (Cr_2O_3; Fisher Scientific Co.) was added daily to the diet. The animals were observed at each feeding until the entire diet was consumed. Approximately 1500 ml of water was given daily. An additional four swine kept in a similar manner were used to evaluate a method for correction of fecal neutral steroid loss. Results with these swine will be described later in the appropriate section.

Experimental design

At the end of 1 wk on mash diet, all 12 animals in the experimental group received a single dose of [4-^{14}C]-cholesterol intravenously and were then put in three dietary groups: mash (MA), mash–cholesterol (MAC), and milk–cholesterol (MKC). All diets contained 100 mg of chromium oxide per day.

In each dietary group two swine were fed for 22 days and the other two received the respective diets for 31 days. Blood samples were obtained at zero time, the following day, and then at 3-day intervals thereafter. Beginning 1 wk prior to killing each animal, blood samples were taken daily. Daily fecal collections were made during the course of the experiment, and total radioactivity and chromium oxide were measured. Feces from the terminal 6 days were used for analyses of fecal steroids and their radioactivity as described below. When the animals were killed, total body cholesterol was obtained by measuring cholesterol concentration in all tissues after complete dissection and weighing of component parts.

Diets

Quantity, composition, estimated caloric value, and additives of the three diets are given in Table 1. Both the MAC and MKC groups received 2 g of crystalline cholesterol daily at the outset. Adjustment for inherent dietary cholesterol content was made midway through the experiment by adding 400 mg of cholesterol to the daily MAC diet, because analysis of the particular milk powder used in the MKC diet indicated that a daily ration contained 400 mg of cholesterol. Thus both MAC and MKC groups received 2400 mg of cholesterol daily, starting 10 days or more before the fecal collection period. The inherent cholesterol content of the mash diet was 23 mg per daily feeding; this small difference was not corrected for.

Isotopes

A single dose of [4-^{14}C]cholesterol (specific activity 47.2 mCi/mmole; Schwarz BioResearch, Inc.) was administered to all 12 experimental swine. 50 μCi of [4-^{14}C]cholesterol was dissolved in 0.5 ml of 100% ethanol and then dispersed in 10 ml of 0.9% saline solution and administered through the anterior vena cava, which was surgically exposed.

All radioactivity measurements were performed using a Packard Tri-Carb liquid scintillation spectrometer, model 3375. After appropriate treatment, samples

TABLE 1. Composition of diets consumed daily

Ingredient	Mash	Mash–Cholesterol	Milk–Cholesterol
		g	
Protein	92.0	92.0	137.5
Fat	23.3	23.3	140.0
Carbohydrate	411.4	411.4	185.0
Fiber or Alphacel	31.5	31.5	100.0
Moisture, minerals, and ash	71.8	71.8	37.5
Chromium oxide	0.1	0.1	0.1
Cholesterol	0	2.0 (1st half) 2.4 (2nd half)	2.4
Total	630.1	632.1 632.5	602.5
Total calories	2224	2224	2550
% Calories as:			
Protein	14.2	14.2	21.6
Fat	9.5	9.5	49.4
Carbohydrate	76.3	76.3	29.0

were put into glass scintillation vials and counts per minute (cpm) were obtained using 15 ml of Bray's solution (18) as scintillator fluid.

Cholesterol determination

Plasma and tissue cholesterol was measured by the method of Leffler (19), using free cholesterol (Sigma Chemical Co.) as a standard. All cholesterol measurements were expressed as free cholesterol.

Fecal collection

Feces were collected on a daily basis as completely as possible during the experiment. Daily collections were weighed and homogenized in a Waring Blendor with a measured volume of water. An aliquot of each homogenate was quickly transferred into a weighed glass container before any settling of solids could occur. The aliquots were dried at 50°C. The dried fecal aliquots were weighed and converted to powders by grinding with a mortar and pestle. Residue powders were stored at −20°C pending analysis.

Total daily fecal output was calculated by measuring recovery of a chromium oxide marker using the method of Davignon, Simmonds, and Ahrens (14). Total fecal radioactivity was initially measured by dissolving 5–10 mg of powdered feces in 0.5 ml of Soluene (Packard Instrument Co.) and then adding scintillator fluid. These measurements were later repeated using a Tri-Carb sample oxidizer, model 305 (Packard Instrument Co.). Results obtained by the two methods were similar.

Analysis of feces collected during terminal 6 days

Correction of fecal neutral steroid loss. In the analysis of feces collected from each animal, the terminal 6 days were divided into two consecutive periods of 3 days each. Fecal residue powders representing fecal outputs from each day of the 3-day periods were pooled in round-bottom flasks and treated as described below. Results obtained from analysis of the two periods were averaged and are presented as one 6-day interval.

In the quantification of fecal neutral steroid material, a major correction is necessary for losses presumed to occur during intestinal transit (15). These losses, when they occur, are believed to be mainly due to bacterial degradation, with the possibilities that volatile end-products may pass out of the body without detection or that the products may be absorbed and stored in body tissues. Evidence has been presented in other studies suggesting that this occurs in man (15) but not in rats (20). Based on the initial report by Gould (21) that absorption of plant sterols in man occurs only to a negligible degree, Grundy, Ahrens, and Salen (15) have proposed that cholesterol losses can be corrected by using the percentage recovery of dietary plant sterols appearing in the feces even if there has been loss as volatile end-products. As an alternative, if there has been no loss as volatile end-products, the extent of neutral steroid degradation can be estimated from the difference between total radioactivity in a fecal aliquot and the sum of radioactivity of neutral and acidic steroids isolated from the aliquot. The validity of using the latter method in this study instead of the plant sterol method was tested in the following preliminary study.

Four male Yorkshire swine approximately 10 wk old and having an average weight of 14.3 kg were put in two groups of two swine each and fed a mash diet for 1 wk. Then one group was given the mash–cholesterol diet and the other the milk–cholesterol diet, both containing 2 g each of crystalline cholesterol and β-sitosterol (Schwarz/Mann) and 100 mg of chromium oxide for the next week. On the 7th day of the experimental diets all four swine received a single dose of 3.13 μCi of β-[22, 23-^{3}H]sitosterol (specific activity 30 Ci/mmole, New England Nuclear) mixed in their diets. The isotope-free experimental diets were then continued for 10 more days, during which total daily fecal collections were made. The feces were processed as described previously, and radioactivity and chromium oxide content were measured in the dried fecal aliquots. Total fecal radioactivities were computed and compared. Recovery of radioactivity in the mash–cholesterol group averaged 95.5% and in the milk–cholesterol group 103.3% (Table 2). Complete recovery of isotope was not part of the original design in the current experiment with isotopic cholesterol. However, after the experiment was completed we decided to determine how much of the given radioactivity could be accounted for in the material that was still available. We had daily fecal samples from all 12 of the Cr_2O_3-fed swine for the entire experimental period.

TABLE 2. Daily recovery of radioactivity in feces after a single feeding of β-[22,23-^{3}H]sitosterol in swine fed mash–cholesterol and milk–cholesterol diets supplemented with 2 g of β-sitosterol

Day	Mash–Cholesterol		Milk–Cholesterol	
	1	2	1	2
	%		%	
1	41.7	32.9	12.2	8.4
2	49.6	45.0	46.5	55.4
3	6.8	11.3	31.4	28.9
4	1.3	6.0	5.4	5.7
5	0.5	2.6	2.3	[a]
6	0.1	1.0	1.1	0.8
7	0.1	0.3	0.8	0.8
8	0	0.8	0.4	0.1
9	0	0.1	0	0
10	0	0	0	0
% Total recovery	99.0	92.0	101.9	104.7

[a] Did not produce feces.

We did not have terminal intestinal contents nor any urine nor epidermal cells sloughed during this period. In spite of these omissions we found in the total feces for the period (Cr_2O_3-corrected), plus radioactivity retained in the carcass, 95%, 84%, and 92% of the original injected radioactivity in the MA group, MAC group, and MKC group, respectively. This provides further evidence that suggests that there had been no significant amount lost in vivo as gas or otherwise. Further study is needed in a variety of experimental situations before it can be determined whether or not [4-^{14}C]cholesterol is ever degraded in the swine to the point that there are significant losses of that portion of the cholesterol molecule containing the radioactivity as gas or as small molecules that might be absorbed in the colon.

From the above results we felt that we could be reasonably certain that, although neutral steroids may be altered during intestinal transit so as to be unrecognizable by chromatographic techniques, most and perhaps all of the products of this degradation remain in the fecal material and can therefore be detected at least by radioactivity measurements. Therefore, we were able to correct for neutral steroid breakdown in the following manner. As described above, total radioactivity per unit weight of dried feces was determined. Since the quantity and specific activities of neutral and acid steroids per unit weight of feces was known by measurement, the total amount of radioactivity accounted for by these identifiable components could be calculated. This amount subtracted from the total amount of radioactivity in the unit weight gave the total radioactivity remaining in the sample not extracted as neutral or acid steroids. As will be described in the Results, in every instance there was a residue of radioactivity not identified as neutral or acid steroids. In a study now in progress we are attempting to characterize the fractions containing the extra radioactivity. These fractions are not in the residue left after complete steroid extraction. They are associated with both the neutral and acid steroid fractions. On thin-layer chromatography some of these fractions stay at the origin while others migrate with the solvent front and are found in spots beyond those identified as neutral or acid steroids by comparison with known standards. This radioactivity was assumed to come from breakdown products of cholesterol, since evidence has been presented in other studies suggesting that bile acids are not broken down during intestinal transit (15). It was also assumed that one molecule of cholesterol had as much chance as any other of being broken down in transit. Thus the specific activity of the cholesterol that had apparently broken down should have been the same as that which came through as intact neutral steroids. By dividing the specific activity of the recognizable neutral steroids into the total radioactivity not accounted for as steroids in a given sample of feces, the amount of neutral steroids represented by breakdown products could be calculated. This value added to that of the recognizable neutral steroids gives the total neutral steroids in the sample. Daily excretion values were determined using the chromium oxide marker as a correction factor.

Fecal neutral and acid steroids were isolated separately, and their masses and specific radioactivities were measured, using methods adapted from those described by Miettinen, Ahrens, and Grundy (5) and Grundy, Ahrens, and Miettinen (6). The following modifications of the general scheme were employed. Methylation of fecal acid steroids in preparation for thin-layer chromatography was performed using diazomethane, prepared according to the method of Shafik and Enos (22). Silylation of both the neutral and acid steroids in preparation for gas–liquid chromatography was accomplished using *N,O*-bis(trimethylsilyl)acetamide ("BSA," Pierce Chemical Co.). Gas–liquid chromatography was performed using a hydrogen flame ionization gas chromatograph (Barber-Colman model 5320) with 6 ft × 4 mm I.D. glass coiled columns. For quantification of neutral steroids the columns were packed with 3% OV-1 on 100–120 mesh Gas-Chrom Q (Applied Science Laboratories). Injector temperature was 275°C, column temperature was 240°C, and detector temperature was 290°C. Nitrogen was used as carrier gas with an inlet pressure of 30 psi. Column efficiency averaged 1800 theoretical plates for the internal standard, 5α-cholestane (Applied Science Labs). For measuring acid steroids the column packing was 3% QF-1 on 80–100 mesh Gas-Chrom Q (Applied Science Labs). Operating temperatures were: injector 270°C, column 225°C, detector 290°C. Nitrogen carrier gas had an inlet pressure of 24 psi. Column efficiency averaged 1200 theoretical plates for the 5α-cholestane standard.

Analysis of gallbladder bile

Concentrations and specific activities of cholesterol and bile acids in gallbladder bile obtained when each animal was killed were determined by the extraction procedure outlined above for fecal neutral and acid steroids. Triplicate aliquots of 1 ml of bile withdrawn from the gallbladder in situ were used for this purpose.

Total body cholesterol analysis

At the termination of the experiment the entire carcass of each swine was dissected into components (organs, tissues, and fluids). Each part was weighed, the cholesterol content of triplicate samples weighing approximately 1 g was determined after extraction of the tissue by the procedure of Folch, Lees, and Sloane Stanley

(23), and values were averaged. Total cholesterol content of the component was then calculated. Specific activity of cholesterol in the extract of each component was also determined.

Estimate of cholesterol retention during the period of fecal analyses (terminal 6 days)

Weight gains of each swine were determined at intervals and plotted on a daily basis. Total body cholesterol concentrations (mg/kg body weight) were determined at the time of killing for each swine, and these provided the basis for the method of estimating retention of cholesterol during the terminal 6 days. The mean for all 12 swine was 737 ± 27 mg/kg, with little variation seen among individual values. The terminal body weights were from 15.7 to 23.4 kg. It is apparent, from the fact that over this weight range the average concentration of cholesterol remains practically the same, that the whole body cholesterol content is directly proportional to body weight. With this point established, daily total body retention of cholesterol could be estimated by multiplying the daily weight gain in kilograms by the terminal cholesterol content per kilogram. Hence, weight in kilograms at sacrifice minus weight 6 days previous multiplied by mean cholesterol content per kilogram gives the total gain in (or net loss from) the body over the 6-day period. This value divided by 6 gives the mean daily retention (or net loss).

Calculation of various parameters of sterol balance

The basis for determining daily excretion of neutral and acid steroids has been explained, as well as the basis for determining retention or net loss. In the mathematical approach which follows, the following system of notation is used: D, daily rate (whole body) of dietary cholesterol input; S, daily rate (whole body) of cholesterol synthesis; R, daily rate (whole body) of cholesterol retention (absolute increment in total body cholesterol); E, daily rate (whole body) of fecal steroid excretion; a, rate of cholesterol absorption from intestine; e, rate of cholesterol excretion into intestine; s, specific activity; c, concentration. Subscripts: p, plasma compartment; b, bile compartment; f, fecal compartment; a, absorbed material; u, unabsorbed material; N, neutral steroids; B, acid steroids (bile acids).

Intake of cholesterol (D) was determined by direct measurement in the diet; all swine, as noted earlier, ate virtually all of the diet containing the cholesterol and chromium oxide marker during the period of study. The dietary cholesterol was not radioactive, so that the total radioactivity in the neutral steroids of the intestine came from excretion of endogenous cholesterol. Thus, the total amount of radioactive cholesterol excreted into the intestine daily was mixed with 2400 mg of nonradioactive dietary cholesterol (D) in the intestine. A portion of the endogenous cholesterol excreted into the gut may have been preferentially reabsorbed. If this portion, which may or may not exist, is excluded from calculations, the mixture in the intestine can be assumed to have the same specific activity as that in the feces (s_{Nf}) obtained by measurement. (Exclusion of the postulated preferentially absorbed neutral steroid must be exactly balanced by an equal amount excreted into the gut, and neither of these are included in the calculations. The only difference the exclusion makes is in the definition of two of the terms. In the text to follow, the term a_N refers to neutral steroid absorbed exclusive of that which may be preferentially reabsorbed. The term e_N refers to the neutral steroid excreted into the gut exclusive of the portion that may be reabsorbed preferentially. To avoid repetitive clumsiness of expression it will be assumed henceforth that the reader understands this limitation of the terms and this aspect will be ignored.) The specific activity of endogenous cholesterol in the feces when it was excreted into the intestine could be assumed to be the specific activity of that in the plasma (s_{Np}) 2 days before the fecal collection (because of transit time). Assuming an intestinal steady state, that is, a condition where neither total nor labeled cholesterol is accumulating in the gut, we can state that the input of cholesterol into the intestinal lumen is balanced by the output of neutral steroids: diet (D) + cholesterol endogenously excreted into the intestinal lumen (e_N) = fecal excretion of neutral steroids (E_N) + absorption of cholesterol (a_N). A similar relationship would be expected for labeled neutral steroids, where counts entering the intestine equal counts leaving the intestine. Expressed mathematically, this relationship takes the form: specific activity of plasma cholesterol (s_{Np}) × e_N = specific activity of fecal neutral steroids (s_{Nf}) × E_N + (s_{Nf} × a_N). In equation form we have the following pair:

$$D + e_N = E_N + a_N$$

$$s_{Np}e_N = s_{Nf}E_N + s_{Nf}a_N$$

Using standard algebraic methods for solving simultaneous equations, e_N and a_N can be calculated as shown in the following equations:

$$e_N = D \times s_{Nf}/(s_{Np} - s_{Nf}) \qquad \text{Eq. 1}$$

$$a_N = D \times s_{Np}/(s_{Np} - s_{Nf}) - E_N \qquad \text{Eq. 2}$$

Knowing the daily amount of cholesterol ingested (D), the daily amount excreted from the body as either neutral or acidic steroids or breakdown products (E), and the daily amount retained (R), the daily amount of cholesterol synthesized (S) can be obtained by difference as follows:

$$S = E + R - D \qquad \text{Eq. 3}$$

If we assume that dietary and endogenous cholesterol excreted into the intestinal lumen are mixed prior to either absorption or excretion in feces, each component of this mixture acquires the specific activity of the fecal neutral steroids (s_{Nf}). Specific activity of fecal neutral steroids (s_{Nf}) = specific activity of plasma neutral steroids (s_{Np}) × unabsorbed endogenously excreted neutral steroids (e_{Nu})/total fecal neutral steroids excreted (E_N), since all the radioactivity appearing in the fecal neutral steroids arose from isotopic cholesterol that was excreted into the intestine and not reabsorbed. We can also state that $s_{Nf} = s_{Np} \times$ endogenous cholesterol absorbed (e_{Na})/total neutral steroids absorbed of both endogenous and dietary origin (a_N) because of the complete mixing between endogenously excreted and dietary cholesterol in the lumen. Furthermore, we know that unabsorbed dietary neutral steroids (D_u) + unabsorbed endogenously excreted neutral steroids (e_{Nu}) = total fecal neutral steroids excreted (E_N). Thus, unabsorbed dietary neutral steroids (D_u) is

$$D_u = E_N(s_{Np} - s_{Nf})/s_{Np} \qquad \text{Eq. 4}$$

Similarly, absorbed dietary neutral steroids (D_a) is given by either of the following equations:

$$D_a = D - D_u \qquad \text{Eq. 5}$$

$$D_a = a_N(s_{Np} - s_{Nf})/s_{Nf}, \text{ since } D_a + e_{Na} = a_N \qquad \text{Eq. 6}$$

Absorbed (e_{Na}) and unabsorbed (e_{Nu}) endogenously excreted neutral steroids are given by the following equations:

$$e_{Na} = a_N - D_a \qquad \text{Eq. 7}$$

$$e_{Nu} = e_N - e_{Na} \qquad \text{Eq. 8}$$

The total amount of bile acids excreted into the intestine and reabsorbed can be calculated, but on more tenuous grounds, since additional assumptions must be made. We measured the amount of neutral and acid steroids in the gallbladder bile, and hence we know their ratio. We can reasonably assume that the two types of steroids are excreted into the intestine via bile in the same ratio. We have already shown how we calculated how much neutral steroid is excreted into the intestine daily. We do not know what percentage of the neutral steroid is excreted via bile compared with the percentage excreted as desquamated intestinal mucosa. In man it has been estimated that 70% is excreted into the gut via the bile (24, 25). If we assume that the 70% value can be applied to the swine in this experiment, the amount of neutral steroids excreted via bile (e_{Nb}) is then known.

$$e_{Nb} = 0.7\ e_N \qquad \text{Eq. 9}$$

Since the ratio of neutral to acid steroids in the bile (c_{Nb}/c_{Bb}) is known by measurement, the daily excretion of acid steroids into the intestine (e_B) can be calculated as follows:

$$e_B = 0.7\ e_N/(c_{Nb}/c_{Bb}) \qquad \text{Eq. 10}$$

Since the amount of acid steroid excreted from the body daily (E_B) is known by measurement, the amount reabsorbed (a_B) is the amount excreted into the intestine minus that excreted from the body.

$$a_B = e_B - E_B \qquad \text{Eq. 11}$$

"Abnormal" retention

It was desired in this study to separate retention of cholesterol due to growth from abnormal retention in the various body tissues. In the control group (MA), concentrations of cholesterol in the tissues usually showed relatively small variability among the four swine. When the corresponding concentrations in the MAC and MKC groups were not significantly different from the MA group for the same tissue, we assumed that there was no abnormal retention of cholesterol and that any change in total quantity was a result of growth. When the cholesterol concentration in a given tissue was significantly higher than that in the control group, we assumed that part of the amount present was abnormal, i.e., attributable to the experimental diet.

The expected normal amount could be estimated by multiplying the weight of the tissue by the concentration of cholesterol for the same tissue in the controls. The difference between this value and the observed value was assumed to represent abnormal accumulation.

Statistical evaluation

The significance of the difference between two means was tested by Student's t test. Significance was ascribed if P was ≤ 0.05.

RESULTS

All swine remained in good health throughout the course of the experiment. They all consumed essentially all of the daily diets shown in Table 1.

Initial and final body weights, final total body cholesterol content and concentration, and increases in body weight and body cholesterol for the terminal 6 days are presented in Table 3. Final mean body weights for the MA, MAC, and MKC groups were 17.9, 17.6, and 21.8 kg, respectively. Overall body weight gains were greater in the MKC group than in either of the other two groups. This difference could have been due in part to a more efficient utilization of the milk than the mash diet, or to the slightly larger caloric content of the MKC diet (Table 1). A similar trend was observed in the gain

TABLE 3. Body weight, total body cholesterol and concentration, and retention of cholesterol in swine fed mash, mash–cholesterol, and milk–cholesterol diets

Group	Swine	Days on Diet	Body Wt		Final Body Cholesterol		Wt Gain for Last 6 days	Body Cholesterol Gain for Last 6 days
			Initial	Final	Total	Concn.		
			kg		*g*	*mg/kg*	*kg/day*	*mg/day*
MA	1	31	13.2	19.3	14.03	727	0.29	211
	2	31	13.4	19.5	14.63	750	0.28	210
	3	22	14.3	15.7	11.18	712	0.23	164
	4	22	11.4	17.0	12.65	744	0.19	141
Average ± SD			13.0	17.9	13.12	733	0.25	182
			±1.21	±1.84	±1.54	±14.1	±0.05	±34.8
MAC	1	31	14.2	19.5	12.83	658	0.02	13
	2	31	14.8	17.5	12.85	734	0.16	117
	3	22	12.3	16.8	12.42	739	0.36	266
	4	22	10.5	16.4	12.01	732	0.23	168
Average ± SD			13.0	17.6	12.53	716	0.19	141
			±1.95	±1.38	±0.40	±37.4	±0.14	±105.4
MKC	1	31	11.7	23.0	17.31	753	0.44	331
	2	31	11.4	23.4	18.05	771	0.49	378
	3	22	12.7	20.9	15.85	758	0.53	402
	4	22	12.3	20.0	15.19	760	0.21	160
Average ± SD			12.03	21.8	16.60	761	0.42	318
			±0.59	±1.64	±1.311	±7.5	±0.14	±109.2

in body weight during the last 6 days of the experiment. The average daily weight gain for the terminal period was 0.25, 0.19, and 0.42 kg for the MA, MAC, and MKC swine, respectively.

After total carcass dissection and measurement of cholesterol in the tissues, the total body cholesterol content of each animal was calculated (Table 3). Average final body cholesterol content for the three dietary groups was found to be 13.12, 12.53, and 16.60 g for the MA, MAC, and MKC groups, respectively. Final total body cholesterol concentrations were 733 ± 14, 716 ± 37, and 761 ± 8 mg/kg, respectively, for the MA, MAC, and MKC groups. These values were essentially the same among the different dietary groups and between swine of the same group that were fed 22 and 31 days.

Average increase in daily total body cholesterol, calculated for the terminal 6 days of the experiment, was found to be 182 ± 35, 141 ± 105, and 318 ± 109 mg/day for the MA, MAC, and MKC groups, respectively. The greater daily retention in the MKC group could be correlated with the larger rate of weight gain in these animals during this period.

The average concentrations of cholesterol (mg/g wet wt) in various organs and tissues obtained after the animals were killed are presented in Table 4. When dietary groups are compared, a similarity in cholesterol concentrations among the groups is seen. The only notable exceptions are plasma, liver, and bone (including marrow) in the MKC group, where concentrations were significantly greater ($P < 0.01$) than in either of the other two groups. The average cholesterol concentration in ileum of this group also appears higher than in the other two groups; this difference however, was not statistically significant.

TABLE 4. Average cholesterol concentrations of various organs and tissues of swine fed mash, mash–cholesterol, and milk–cholesterol diets

	MA	MAC	MKC
	mg/g ± SD[a]		
Plasma	0.91 ± 0.12	0.81 ± 0.13	1.94 ± 0.41[b]
Spinal cord	33.88 ± 7.91	34.88 ± 5.09	31.79 ± 5.88
Brain	15.64 ± 1.15	15.25 ± 1.65	17.30 ± 2.37
Spleen	3.65 ± 0.58	3.62 ± 0.42	3.17 ± 0.88
Lung	3.01 ± 0.58	2.71 ± 0.18	3.06 ± 0.60
Adrenal	3.06 ± 0.64	2.84 ± 0.19	2.80 ± 0.53
Kidney	2.72 ± 0.43	2.72 ± 0.19	2.80 ± 0.53
Testes	2.31 ± 0.12	2.16 ± 0.27	2.42 ± 0.23
Stomach	2.14 ± 0.25	1.84 ± 0.21	1.81 ± 0.25
Liver	1.99 ± 0.09	1.85 ± 0.04	2.93 ± 0.33[b]
Jejunum	1.88 ± 0.21	1.61 ± 0.21	1.81 ± 0.25
Pancreas	1.87 ± 0.15	1.85 ± 0.16	1.71 ± 0.14
Colon	1.78 ± 0.44	1.54 ± 0.11	1.82 ± 0.32
Duodenum	1.76 ± 0.27	1.78 ± 0.22	1.67 ± 0.33
Rectum	1.59 ± 0.08	1.58 ± 0.11	1.35 ± 0.19
Muscle	0.57 ± 0.03	0.58 ± 0.04	0.49 ± 0.00
Ileum	1.54 ± 0.08	1.56 ± 0.17	1.99 ± 0.36
Urinary bladder	1.31 ± 0.31	1.27 ± 0.01	1.29 ± 0.26
Thyroid	1.09 ± 0.13	1.13 ± 0.22	1.13 ± 0.10
Heart	1.05 ± 0.17	1.02 ± 0.07	1.06 ± 0.13
Skin	0.99 ± 0.18	0.83 ± 0.12	0.92 ± 0.10
Esophagus	0.93 ± 0.12	0.95 ± 0.06	0.99 ± 0.07
Aorta	0.80 ± 0.08	0.89 ± 0.12	0.99 ± 0.32
Adipose tissue	0.64 ± 0.14	0.60 ± 0.11	0.57 ± 0.08
Bone	0.13 ± 0.01	0.12 ± 0.02	0.24 ± 0.06[b]

[a] Wet weight.

[b] Plasma, liver, and bone including bone marrow values are significantly higher in MKC than in either of the other two groups, $P < 0.01$.

The average cholesterol contents (tissue cholesterol

concentration × total tissue weight) of the various organs and tissues of swine comprising the three dietary groups are shown in Table 5. The following points are noteworthy for all three groups: (*1*) brain and spinal cord, which supposedly contain "nonexchangeable" body cholesterol, contribute approximately 10% to the total body cholesterol; (*2*) the remaining 90% is accounted for largely by a few tissues: muscle, gastrointestinal tract, adipose tissue, skin, blood, and liver; (*3*) those tissues in which cholesterol is considered to be readily equilibrated with plasma cholesterol (red cells, liver, spleen, kidney, lung, and intestines) constitute 36.9% of the total body cholesterol, while the slowly exchanging pool accounts for 53.4% of the cholesterol in the body. Comparisons among the three dietary groups reveal a greater total cholesterol content in most tissues from swine fed the high-fat cholesterol diet (MKC) as compared with the low-fat cholesterol (MAC) and control (MA) groups. The greater absolute amounts of cholesterol in the MKC group are believed to reflect a greater rate of body growth for many of the tissues plus an increased rate of deposition of cholesterol in a few tissues.

In the Methods section we outlined a basis by which we were able to evaluate the cholesterol concentrations in the various body tissues in an attempt to identify those tissues in which the average concentrations of cholesterol were significantly greater then control levels. As stated above in reference to Table 4, those tissues were found to be plasma, liver, and bone with marrow of the MKC group. A comparison was made between the total cholesterol contents actually observed and the contents which would be expected if these tissues had cholesterol concentrations similar to those found in the control animals. On this basis (observed vs. expected) we found for plasma (1452 mg vs. 708 mg) an increase of 105.1%, for liver (1306 mg vs. 778 mg) an increase of 67.9%, and for bone marrow (798 mg vs. 436 mg) an increase of 83.6% above control levels. In all other body tissues of the MKC animals, total cholesterol contents were found to be greater than control values, but these differences could be totally accounted for by the greater mass of the tissues. Only in plasma, liver, and bone marrow were the total cholesterol contents significantly greater than what could be attributed to the greater masses. These tissues, then, are believed to reflect sites of abnormal cholesterol retention in the MKC swine.

The specific activities of cholesterol in plasma and in all body tissues obtained at killing were compared. Table 6 shows the average specific activities of plasma cholesterol (at time of killing) for all three dietary groups and the ratio (relative specific activity) between tissue and plasma cholesterol in each of these groups. As can be seen, several body tissues had terminal cholesterol spe-

TABLE 5. Average cholesterol contents of various organs and tissues of swine fed mash, mash–cholesterol, and milk–cholesterol diets

	MA	MAC	MKC
		mg	
Muscles	3828.4	3714.4	4386.7
Gastrointestinal tract	2035.0	2114.9	2418.2
Adipose tissues[a]	1367.0	1200.9	1406.5
Skin	1272.7	1019.0	1439.4
Brain	1116.9	1120.2	1289.7
Blood	802.5	740.5	1796.5
Liver	762.8	730.6	1308.0
Spinal cord	680.1	706.0	666.7
Lungs	428.3	386.8	493.5
Bones[b]	347.3	331.3	797.1
Kidneys	185.2	174.2	233.1
Spleen	106.8	101.3	109.1
Heart	74.6	70.9	100.5
Pancreas	49.5	45.7	56.1
Testes	37.5	45.2	63.3
Urinary bladder	13.3	13.0	15.4
Aorta	7.9	9.3	11.4
Adrenals	4.7	4.4	5.7
Thyroid	1.6	1.7	2.0
Total	13,122.1	12,530.3	16,598.9
Average body wt (kg)	17.9	17.6	21.8
Average body cholesterol concentration (mg/kg)	773	716	761

[a] Includes mesentery.
[b] Includes marrow.

TABLE 6. Ratio of average specific activity of cholesterol in tissues at autopsy to that of plasma (22–31 days after isotope injection)

Tissue	MA	MAC	MKC
Plasma[a]	1.00	1.00	1.00
Bile	1.01	0.92	1.01
Jejunum	1.05	1.25	0.84
Colon	1.13	1.29	1.23
Spleen	1.14	1.15	1.06
Ileum	1.21	1.37	0.92
Testes	1.27	1.57	1.19
Duodenum	1.36	1.38	1.10
Rectum	1.36	1.48	1.34
Liver	1.39	1.56	1.15
Stomach	1.51	2.10	1.45
Heart	1.61	1.62	1.29
Adrenal	1.67	1.64	1.30
Thyroid	1.70	1.91	1.45
Skin	1.75	2.07	1.45
Kidney	1.92	2.30	1.65
Urinary bladder	1.97	2.35	1.69
Esophagus	1.99	1.98	1.48
Muscle	2.10	2.58	1.73
Aorta	2.19	2.71	1.63
Adipose tissue	2.22	3.19	1.96
Pancreas	2.35	2.50	1.79
Lung	6.32	8.79	5.68
Bone	0.12	0.11	0.06
Brain	0.09	0.12	0.07
Spinal cord	0.03	0.03	0.03

[a] Terminal plasma specific activity (dpm/mg cholesterol): MA, 601; MAC, 458; MKC, 1122.

cific activities which closely approximated that of the plasma (i.e., the ratio is close to 1.00), while in other tissues the specific activity of cholesterol was much greater than in plasma. In those tissues in which cholesterol exchange with the plasma is considered to be extremely slow or nonexistent (bone, brain, spinal cord), cholesterol specific activities were considerably lower than the plasma.

Fig. 1 presents graphically the average changes in plasma cholesterol in the three dietary groups. The left-hand graph represents plasma cholesterol levels in swine fed diets for 31 days; the right-hand graph is for 22 days of feeding. In both cases we found that plasma cholesterol in the MKC swine became markedly elevated after 1–2 days on diet and then remained somewhat stable at the new levels. In the MAC group, it can be seen that the addition of cholesterol to the diet produced no change in plasma cholesterol levels for the duration of the experiment.

The various parameters of cholesterol metabolism in swine comprising the three dietary groups studied in this experiment were obtained through analysis of the feces collected during the terminal 6 days of the experiment. The raw data are presented in Tables 7 and 8. In every instance, total fecal radioactivity was greater than the sum of the radioactivities identified as neutral and acid steroids from a similar aliquot (Table 7). The unidentified radioactivity, presumed to be a product of neutral steroid degradation, indicates that from 36.8 to 54.3% of the total fecal neutral steroids have undergone conversion during intestinal transit, presumably mediated by intestinal bacteria. Measurement of neutral steroids (cholesterol, coprostanol, and coprostanone) in an aliquot of dry material from each 3-day pool enables one to calculate the total amount of neutral steroids excreted daily which were recognized by gas–liquid chromatography (column 1, Table 8). The amount of fecal neutral steroids excreted daily in the control group (MA) was small when compared with that of swine on the other diets, since the MA swine received no dietary cholesterol. Fecal neutral steroid excretion in this group, then, represents only material entering the intestine from either the bile or intestinal mucosa. For the MAC and MKC groups, both of which received dietary cholesterol, fecal neutral steroids represent material from exogenous and endogenous sources. The sum of neutral steroids observed and degraded (columns 1 and 2 of Table 8) equals the total amount of neutral steroids excreted daily during the 6-day period. Total daily neutral steroid excretion was greater in the MAC group than in the MKC group, even though dietary intake of cholesterol was the same for both groups. The greater excretion is seen both in the amounts of neutral steroids actually measured and in the amounts presumably lost through degradation.

Excretion of fecal bile acids was also measured on a daily basis for the terminal 6 days; no correction for bile

TABLE 7. Average fecal radioactivity excreted during terminal 6 days of study corrected for fecal flow with chromium oxide

Group	Swine No.	Total	NS[a]	BA[a]	Unidentified[b]
		dpm/day			
MA	1	1,018,590	294,120	551,610	172,860
	2	475,290	106,196	285,138	83,956
	3	855,525	168,720	593,325	94,480
	4	460,105	124,266	241,522	94,317
MAC	1	416,757	75,756	251,026	89,975
	2	431,868	89,579	243,530	98,758
	3	1,142,924	242,900	643,824	256,200
	4	867,894	135,762	587,292	144,840
MKC	1	663,728	184,950	371,096	107,682
	2	994,800	153,760	672,880	168,160
	3	1,180,047	349,920	504,507	325,620
	4	1,029,653	261,856	523,861	243,936

[a] Radioactivity present in the chromatographically identifiable neutral (NS) and acid (BA) steroids.
[b] Unidentified = total radioactivity − (NS + BA).

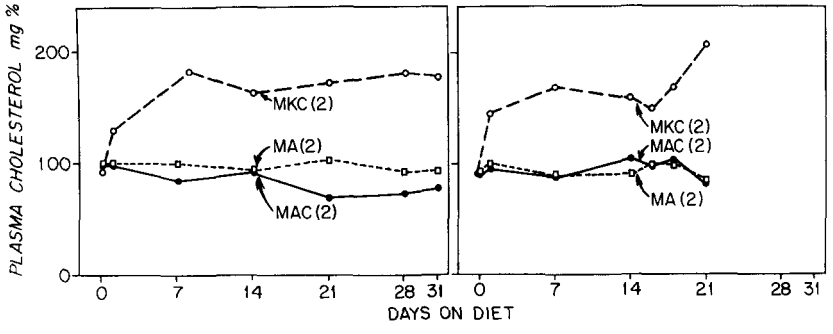

FIG. 1. Plasma cholesterol levels (mg/100 ml) of swine fed mash (MA), mash–cholesterol (MAC), and milk–cholesterol (MKC) diets. Left panel illustrates changes in average plasma cholesterol of two swine from each dietary group on diets for 31 days. Right panel illustrates similar changes in swine fed diets for 22 days. In both cases plasma cholesterol in the milk–cholesterol swine became markedly elevated after 1–2 days on diet. In the mash–cholesterol group, dietary cholesterol produced no increase in plasma cholesterol over control (MA) levels.

TABLE 8. Data on steroids of feces and plasma based on terminal 6 days, and terminal steroid concentrations of gallbladder bile

Group	Swine No.	Feces: NS[a] Observed	Feces: NS[a] "Degraded"[b]	Feces: NS[a] Total	Feces: BA[a] Total	Feces: Total Steroids (NS + BA)	Feces: Spec. Act. NS	Plasma Spec. Act. (s_{Np})	Gallbladder Bile: Cholesterol	Gallbladder Bile: BA
		mg/day			*mg/day*	*mg/day*	*dpm/mg*	*units*	*mg/ml*	
MA	1	456	268	724	227	951	645	1005	0.98	42.56
	2	191	151	342	279	621	556	626	1.18	38.74
	3	296	164	460	293	753	570	760	1.57	57.25
	4	278	211	489	197	686	447	524	1.37	36.52
Avg. ± SD		305	199	504	249	753				
		±110	±53	±160	±45	±143				
MAC	1	1284	1525	2808	313	3121	59	349	0.86	57.06
	2	1337	1474	2811	245	3056	67	421	1.66	52.91
	3	1388	1464	2852	272	3124	175	746	1.94	70.80
	4	1331	1420	2751	436	3187	102	582	1.88	71.67
Avg. ± SD		1335	1471	2806	317	3122				
		±43	±43	±42	±84	±53				
MKC	1	1350	786	2136	88	2224	137	867	1.28	36.71
	2	961	1051	2012	80	2092	160	992	1.85	43.51
	3	1080	1005	2085	57	2142	324	1907	0.97	48.91
	4	1169	1089	2258	59	2317	224	1590	1.49	47.48
Avg. ± SD		1140	983	2123	71	2194				
		±164	±136	±104	±15	±82				

[a] NS and BA, neutral and acid steroids, respectively.
[b] Derived from "unidentified" radioactivity in feces as described in text and presented in Table 6.

acid loss was employed, since Grundy et al. (15) have indicated that bile acids are not lost during intestinal transit. The amount of bile acids appearing daily in the feces was relatively small in the MKC group when compared with either the MAC group or the MA group. In the latter two groups, daily bile acid excretion rates were somewhat comparable, even though the MAC group received 2400 mg of cholesterol daily and the MA group did not.

The sum of total neutral steroids and bile acids expresses total daily steroid excretion (column 5, Table 8). Between the two groups receiving dietary cholesterol (MAC and MKC), total daily steroid excretion was significantly greater in the MAC group ($P < 0.005$). On the average, daily excretion of steroids by animals in the MAC group was 900 mg greater than that calculated for animals in the MKC group. This increased excretion was reflected in both the neutral and the acid steroid fractions of the feces.

The calculations based on these data make use of the specific activity of plasma cholesterol. Because of the lag between the time when neutral steroids enter the gut and when they appear in the feces, plasma cholesterol specific activity was corrected by averaging daily specific activities observed 2–8 days prior to sacrifice of each animal. These averaged values are given in Table 8.

Table 9 gives the calculated results obtained when the data presented in Table 8 are utilized in conjunction with the series of equations presented above in the Methods section. These results are presented on an average per diem basis for the entire 6-day period. Thus, E_N, E_B, and E in Table 9 are the average daily values for excretion of neutral, acid, and total fecal steroids, respectively, during the 6-day interval. Retention (R) represents daily retention of cholesterol during the time these feces were collected. As discussed above for Table 3, the average increase in body cholesterol during this period was greatest for the MKC swine, which also showed the greatest rate of daily weight gain.

From Eq. 3 in Methods we can obtain daily synthesis (S) of cholesterol by the difference: total steroid excretion (E) + retention (R) − dietary intake (D). Average daily cholesterol synthesis in the MAC animals was calculated to be 865 mg; this value is not significantly different from the rate of synthesis in the control animals (MA, 916 mg/day), which did not receive dietary cholesterol. In the MKC group, however, average daily synthesis was calculated to be 111 mg; this value is significantly lower than rates for both the MA and MAC groups ($P < 0.005$). The relative importance of this difference in rates of cholesterol synthesis will be seen when a comparison of values for daily cholesterol absorption is made below.

The amount of endogenous cholesterol excreted daily into the intestine (e_N) via the bile and intestinal mucosa is obtained from Eq. 1. Plasma cholesterol specific activity represents the value obtained by averaging specific activities found 2 days prior to each daily fecal

TABLE 9. Neutral, acid, and total fecal steroids, total body retention and synthesis of cholesterol, plus absorption and endogenous excretion of neutral and acid steroids[a]

Group	Swine No.	Days on Diet	E_N	E_B	E	R	S	e_N	a_N	D_u	D_a	e_{Nu}	e_{Na}	e_B	a_B
MA	1	31	724	227	951	211	1139								
	2	31	342	279	621	210	808								
	3	22	460	293	753	164	894								
	4	22	489	197	686	141	804								
Avg. ± SD			504	249	753	182	911								
			±160	±45	±143	±35	±157								
MAC	1	31	2808	313	3121	13	734	488	80	2332	68	476	12	22,623	22,310
	2	31	2811	245	3056	117	773	454	43	2365	35	446	8	10,121	9,876
	3	22	2852	272	3124	266	990	735	284	2183	217	668	67	18,777	18,505
	4	22	2751	436	3187	168	955	510	159	2268	132	483	27	13,626	13,190
Avg. ± SD			2806	317	3122	141	863	547	142	2287	113	518	29	16,287	15,970
			±42	±84	±53	±105	±128	±128	±107	±80	±80	±101	±27	±5521	±5522
MKC	1	31	2136	88	2224	331	155	450	714	1797	603	339	111	9,026	8,938
	2	31	2012	80	2092	378	70	462	849	1688	712	325	137	7,609	7,529
	3	22	2085	57	2142	402	144	491	806	1732	668	353	138	17,185	17,128
	4	22	2258	59	2317	160	77	393	536	1940	460	317	76	8,761	8,702
Avg. ± SD			2123	71	2194	318	112	449	726	1789	611	334	116	10,645	10,574
			±104	±15	±82	±109	±44	±41	±139	±110	±110	±16	±29	±4403	±4412

[a] All values are expressed in mg/day for terminal 6 days. E_N and E_B, neutral and acid steroids excreted from body; $E = E_N + E_B$; R, daily increment of retained cholesterol; S, synthesized cholesterol; e_N, endogenous neutral steroids excreted into gut; a_N, sum of endogenous and dietary cholesterol absorbed; D_u and D_a, dietary cholesterol unabsorbed and absorbed; e_{Nu} and e_{Na}, endogenous unabsorbed and absorbed cholesterol; e_B, bile acids excreted into gut; a_B, reabsorbed bile acids.

collection (i.e., 2–8 days prior to killing). Since dietary input in the MA swine was almost negligible, the parameters of endogenous cholesterol excretion into the intestine, as well as absorption from the intestine (a_N), could not be accurately determined. In comparing daily endogenous excretion of cholesterol between the two groups that received dietary cholesterol, we see that the amount is somewhat greater in MAC than in MKC (average values 547 mg vs. 449 mg), but this difference is not statistically significant. In other words, the rates at which cholesterol entered the intestinal lumen in swine comprising these two groups were similar.

The absorption of neutral steroids from the intestine (a_N) can be calculated independently by Eq. 2. Calculation of daily cholesterol absorption revealed a striking difference between the two dietary groups receiving cholesterol. In the MAC group, average daily cholesterol absorption was determined to be 142 mg/day, while in the MKC group this value was much greater, the average being 726 mg/day (difference significant at $P < 0.005$). We thus see that in the MAC group, where synthesis of cholesterol was high, absorption of cholesterol is small. The converse is seen for the MKC group, for which rates of synthesis were low but rates of cholesterol absorption were high. These points will be discussed in more detail in the Discussion.

The term for absorption of neutral steroids (a_N) includes both dietary cholesterol and cholesterol that has reached the intestinal lumen from the bile and intestinal mucosa. Since we wished to consider separately that portion of cholesterol entering the gut from the diet, Eq. 4–6 in the Methods were utilized. In Table 9 we see a marked difference between the MAC and MKC swine with regard to the amount of dietary cholesterol absorbed daily (D_a). In the MAC group, average daily absorption of dietary cholesterol was calculated to be 113 mg, or 4.7% of the total amount available for absorption (2400 mg). In MKC, on the other hand, average absorption of cholesterol from the diet was 611 mg/day, or 25.5% of the total.

Once we know the value of absorbed dietary cholesterol (D_a), we can calculate what portion of the endogenously excreted neutral steroids is absorbed (e_{Na}) (Eq. 7), since we know the value of total absorption. Similarly, Eq. 8 enables us to calculate by difference unabsorbed neutral steroids endogenously excreted (e_{Nu}). We see in Table 9 that a somewhat greater portion of the endogenously excreted neutral steroids entering the intestine in MAC swine is unabsorbed as compared with neutral steroids in the MKC swine. In addition, Table 9 shows that the daily rate of excretion of bile acids from the body was considerably less in the MKC than in the MAC and MA groups. This presumably means that bile acids are more completely reabsorbed in the MKC group than in the others.

DISCUSSION

In any animal species, total body cholesterol is maintained at constant levels only when the counterbalancing

mechanisms of absorption, synthesis, and excretion of cholesterol are in harmony. Any deviation will eventually result in either an expansion or reduction in the tissue pools of cholesterol. Different species of laboratory animals have been found to vary considerably in the interplay of these compensatory mechanisms. For example, feeding cholesterol to rabbits causes a marked increase in the amount of cholesterol in blood and tissues because the rate of absorption of cholesterol far exceeds the rate of its removal (26). In rats, on the other hand, cholesterol feeding causes only small increases in cholesterol concentrations in tissues other than the liver (27). This species has been shown to compensate for increased absorption of dietary cholesterol by a marked inhibition of cholesterol synthesis (28) plus an increased rate of excretion of bile acids (29). With regard to man, the interrelationships between absorption, synthesis, and excretion of cholesterol are currently subjects of extensive study. Contrary to earlier opinions, recent studies have shown that man has the ability to suppress synthesis of body cholesterol, although the extent of suppression may vary markedly among individuals. For example, Quintão, Grundy, and Ahrens (12) have recently observed that increased cholesterol absorption evoked moderate to almost complete reductions in body cholesterol synthesis in some of their patients, while other patients studied failed to show any suppression of synthesis. In addition, it has been calculated that increased cholesterol absorption results in a 50% suppression of cholesterol synthesis in the Masai of East Africa (9).

A comparison of overall cholesterol balance between the low-fat cholesterol group (MAC) and the high-fat cholesterol group (MKC) is presented schematically in Fig. 2. Each arrow represents an input or output of cholesterol expressed in milligrams per day. All values are the average for each parameter among the swine in a given dietary group. In swine on the low-fat diet (MAC), an average of only 4.7% (113 mg) of the 2400 mg of dietary cholesterol was absorbed daily. Swine fed

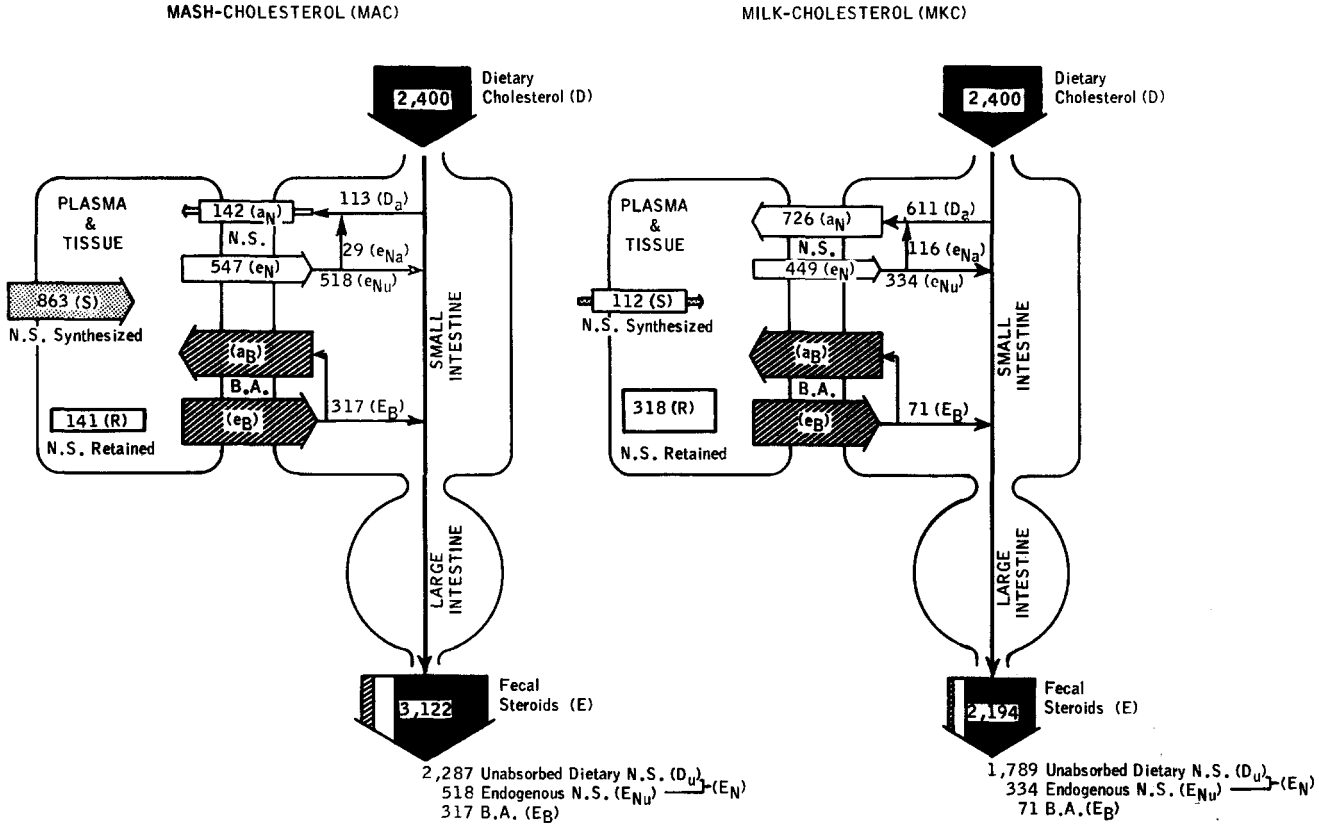

FIG. 2. Overall cholesterol balance in swine fed mash–cholesterol (MAC) and milk–cholesterol (MKC) diets. Each arrow represents an input or output of cholesterol (and its steroid metabolites) expressed in mg/day. Values shown are the average for four swine in each dietary group. Dietary cholesterol input (D) was the same for both groups (2400 mg). Absorption of dietary cholesterol (D_a), as well as absorption of cholesterol excreted into the gut (e_{Na}), was significantly greater in the MKC group. Thus, total neutral steroid absorption (a_N) also was significantly greater in MKC than in MAC. In addition, cholesterol retention (R) was greater in MKC. In the MAC group, where absorption and retention of cholesterol was low, daily cholesterol synthesis (S) approached rates for control animals, which received no dietary cholesterol, and was significantly greater than values obtained for the MKC group. Fecal excretion of steroids (E) was significantly greater in the low-fat group (MAC). The greater excretion included unabsorbed dietary cholesterol (D_u) and endogenously excreted neutral steroids (E_{Nu}) and bile acids (E_B).

the high-fat diet (MKC), on the other hand, absorbed daily an average of 25.5% (611 mg) of the 2400 mg of dietary cholesterol. Similar influences of dietary triglycerides on cholesterol absorption have been found in all experimental animal species studied as well as in man. Quintão et al. (12), for example, have recently found that the feeding of crystalline cholesterol to a patient for 1 month in conjunction with a fat-free diet resulted in none of the dietary cholesterol being absorbed.

An interesting correlation was found between cholesterol absorption and synthesis in the swine studied in this report. The average rate of synthesis of cholesterol was found to be depressed in the high-fat group (MKC; 112 mg/day), whereas the addition of cholesterol to the low-fat diet (MAC; synthesis 863 mg/day) did not significantly alter the rate of cholesterol synthesis from that in the control animals (MA), which received no dietary cholesterol. The fact that the absorption of dietary cholesterol was almost negligible in the low-fat group, but comparably much greater in the high-fat group, indicates that swine are able to compensate to a considerable extent for increased absorption of cholesterol by depressing the rate of sterol synthesis. Swine, then, resemble humans in this respect. By comparison with the rates of cholesterol synthesis in the control animals (MA; synthesis 911 mg/day), we have estimated that an 88% suppression of synthesis occurred in the high-fat group. We were not able to ascertain, however, whether the remaining 12% of total body synthesis in this group is attributable totally, or only in part, to extrahepatic synthesis. It is interesting to note that in the squirrel monkey, extrahepatic tissues, especially the small intestine, account for 10–15% of total body cholesterol synthesis under normal conditions (30).

Excretion of endogenous neutral steroids into the intestine was not significantly different in the low- and high-fat cholesterol groups (547 vs. 449 mg/day). Excretion of endogenous neutral steroids from the body by way of the feces, however, was somewhat less in the high-fat cholesterol group than in the low-fat cholesterol group (334 vs. 518 mg/day). Excretion of bile acids in the feces was found to be considerably less in the high-fat group (71 mg/day vs. 317 mg/day for MAC). In one study with humans, increased absorption of cholesterol in a moderately high-fat diet has been associated with an increased fecal output of endogenous neutral steroids and no significant change in fecal output of acidic steroids (12). A second study in man (31) has indicated that a low-fat, high-carbohydrate diet resulted in markedly increased bile acid excretion (403 mg vs. 292 mg on a high-fat diet). This aspect needs to be explored further both in man and in experimental animals.

Among the aims of this study was an attempt to develop means for studying retention of cholesterol in the body. The total body cholesterol content of laboratory animals can be measured directly at the termination of any given experiment. No assumptions related to various pool concepts are necessary. Cholesterol retention over finite intervals, however, cannot be measured directly, and total body cholesterol at any point prior to termination of the experiment can only be estimated. The most practical way to estimate total body cholesterol in living animals, such as those utilized in this experiment, would be to relate it to body weight. This method, however, is reliable only if two conditions are met. First, body weight has to be proportional to the weights of specific tissues that contain most of the cholesterol. Table 3 shows that, within the range of body weight in the current experiment, this condition was met. Most of the weight differences are accounted for by increases in muscle, skin, adipose tissue, bone, and blood, which constitute approximately 80% of the total body weight. Secondly, the cholesterol concentrations of all (or at least most) tissues should not change significantly with changes in weight. Table 4 shows that this was true within each of the three groups even though there were a few differences among groups. Needless to say, this generalization should not be applied to weight ranges other than that which we have observed in this study without further testing. The close relationship between body weight and total body cholesterol content regardless of the diet was a reflection of the relative constancy of cholesterol concentration in the tissues that are major pools of cholesterol such as skin, muscle, and adipose tissue. Obviously, plasma cholesterol levels were higher in the high-fat cholesterol (MKC) group. However, plasma concentrations in swine of this group did not show any significant changes during the final 6-day period. Furthermore, plasma cholesterol constitutes less than 10% of total body cholesterol. Thus, no special treatment seemed necessary in computing estimated total body cholesterol of swine 6 days prior to killing.

An approach to the determination of cholesterol retention in man similar to that in swine would be possible only if man showed the same close relationship between body weight and total body cholesterol as do swine. We know that in advanced atherosclerosis the cholesterol concentration in the aorta and its branches increases manyfold over normal amounts (13). Even so, the amount of cholesterol in the arteries represents a relatively small part of the total in the body, and it probably has accumulated over a period of many years. Thus, the change over weeks or even months would be expected to be quite small. The crucial question is whether or not there are wide fluctuations with time in the cholesterol concentration in the other tissues in the body. Maurizi et al. (32) recently measured cholesterol concentrations in various tissues obtained from 46 human autopsies on

individuals ranging in age from 1 to 102 yr. Cholesterol concentration in the various tissues that were examined showed only moderate variability. There was no significant relationship of cholesterol concentration to age except that concentration in muscle was somewhat greater in the young. Also, those with the most atherosclerosis (average age 79.6 yr) failed to show higher concentrations in any of the tissues that were studied than did those with the least atherosclerosis (average age 20.6 yr), except in the arteries. Their report suggests that man has little ability to sequester significant amounts of cholesterol in tissues other than arteries. Hence, the relationship between body weight and total body cholesterol might be as constant as in the swine in the current study. Such a conclusion, however, should be questioned somewhat when one deals with individuals exhibiting hyperlipemias of genetic origin. For example, Quintão et al. (12) found that large amounts of cholesterol were retained in patients with type II hyperlipidemia, a genetic condition frequently associated with gross deposits of cholesterol in the skin and tendons. More recently, Bhattacharyya, Connor, and Spector (33) have found slower turnover rates plus expansion of the readily exchangeable pool of body cholesterol in patients having this type of hyperlipidemia. These findings certainly suggest that storage of cholesterol in this type of genetically altered individual may differ from that occurring in the more normal situation.

Marked differences have been noted among various species with regard to tissue storage of cholesterol in sites other than arteries. Hypercholesterolemic rhesus monkeys (34) and rabbits (35) are known to store large amounts of cholesterol in many body tissues. In hypercholesterolemic rats excess storage is largely limited to the liver (27, 35). In the hypercholesterolemic swine of the current study, cholesterol concentration in the liver was moderately increased. The question arises whether the cholesterol concentrations in body tissues might have shown larger and more widespread increases had the period of hypercholesterolemia been longer than 31 days. That this does not appear to be the case has been shown in an earlier study[1] where cholesterol concentrations in various body tissues of swine that were hypercholesterolemic for 200 days were determined. The only significant increase observed in any component except plasma and arteries was in the liver, and this was moderate. Thus it appears that, even after prolonged periods of hypercholesterolemia in swine, increase in concentration of cholesterol in extravascular portions of the body does not occur to any significant extent.

In man, total body cholesterol has been considered to conform to a two-pool model (3). According to Chobanian and Hollander (1) and Field et al. (36), cholesterol in several body tissues equilibrates rapidly with radioactive plasma cholesterol. The other body tissues, excepting the central nervous system, are slow to equilibrate with the plasma cholesterol, requiring up to 1 month for equilibration (1, 36). In the current study we have observed that most of the body tissues of the growing swine had cholesterol specific activities that were considerably greater than that in the plasma, even at 31 days. A possible explanation for this discrepancy with comparable studies in humans (1, 36) is that in our case young, growing animals were used whereas in the human cases the subjects were terminally ill adults. After the injection of [4-^{14}C]cholesterol into our swine, the isotope may have been incorporated into structural elements of the growing tissues at a time when plasma cholesterol specific activity was near its peak value. Subsequent exchange with the plasma might then be quite slow, leaving those tissues with high specific activities relative to plasma cholesterol. We may also be dealing with a species difference when we consider swine. In any case, this fact regarding plasma–tissue cholesterol exchange would make it quite difficult to apply the two-pool model concepts of tissue cholesterol pools in man to our situation with growing swine.

In the course of our chemical analyses, we observed that the sum of the radioactivity isolated from the feces as neutral plus acid steroids was less than the total fecal radioactivity in every instance. The assumption was made that the radioactivity unaccounted for represented breakdown products arising from the neutral steroid fraction, and the appropriate corrections for this breakdown were made as described above under Methods. Studies with humans, in which sitosterol was used as a marker for neutral steroid degradation, have indicated that, although total recovered fecal radioactivity can be accounted for by neutral plus acid steroids, variable amounts of sitosterol are lost during intestinal transit (15). This would suggest that at least in man certain portions of the fecal neutral steroids have become altered to the extent that they are lost to any means of detection in the feces (for example, as gaseous material). The question thus arises whether one can reliably make use of the total amount of radioactivity measured in the feces to correct for neutral steroid losses in swine. As we have indicated under Methods, when swine in a preliminary experiment were fed the types of diets utilized in these experiments, almost 100% of the radioactivity initially entering the body as sitosterol eventually was recovered from the feces (either as sitosterol or its bacterial conversion products) when the feces were collected for a sufficient period of time, in this case 10 days. Since none of the radioactivity was lost, we postulated that a similar situation would exist with regard to the recovery of

[1] Marsh, A. C., and D. N. Kim. Unpublished data.

radioactive material originating as cholesterol. We are currently conducting experiments to compare directly in the same swine the β-sitosterol recovery technique with the radioactivity procedures used for estimating neutral steroid losses in the present study.

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