

Cholesterol absorption and steroid excretion in cholesterol-fed guinea pigs

Maret G. Traber¹ and R. Ostwald²

Department of Nutritional Sciences, University of California, Berkeley, CA 94720

Abstract Cholesterol absorption was studied in groups of guinea pigs fed diets containing 0, 0.1%, or 1% cholesterol. A similar proportion of tracer cholesterol was absorbed regardless of the cholesterol content of the diet. Furthermore, the proportion of tracer cholesterol absorbed by individual animals did not change when the cholesterol-free diet was changed to one containing 1% cholesterol.

Cholesterol absorption was also measured in hypo-responding guinea pigs. These guinea pigs had been fed 1% cholesterol-containing diets for nearly a year with minimal pathological effects. These hyporesponders had a decreased intestinal transit time, which enabled them to decrease the fractional absorption of cholesterol below the levels seen in the controls, and to absorb less cholesterol/kg body weight than the hyperresponders.

Excretion of total and of neutral steroids was measured in guinea pigs fed 0 or 1% cholesterol-containing diets. The 1% cholesterol-fed guinea pigs increased the excretion of steroids 3-fold over control levels. However, they absorbed more dietary cholesterol than they excreted in any form. It seems, therefore, that a major cause of the cholesterol pool expansion in the guinea pig is its inability to limit absorption of dietary cholesterol in conjunction with its inability to sufficiently increase excretion of steroids.

Supplementary key words cholesterol pool · cholesterol balance · cholesterol metabolism · bile acid excretion · plant sterols · aging · cholesterol hyporespondence

Guinea pigs, in response to a diet containing 1% cholesterol, become hypercholesterolemic and, eventually, develop a fatal hemolytic anemia (1–3). In contrast, guinea pigs fed 0.1% cholesterol do not become hypercholesterolemic, or anemic (4). Both 1% and 0.1% cholesterol-fed guinea pigs, however, exhibit extensive expansions of their body cholesterol pools (1, 2, 4–6).

There are, theoretically, three mechanisms to control the expansion of the cholesterol pool: 1) decreased absorption of dietary cholesterol, 2) increased excretion of either, or both, cholesterol and its metabolites—the bile acids; and 3) decreased synthesis of cholesterol (7). Two of these mechanisms to

limit cholesterol pool expansion have been shown to operate to some extent in the guinea pig. Bile acid excretion is increased at least 3-fold in response to a 1% cholesterol-containing diet (8), and cholesterol synthesis is decreased in response to a diet containing 5% cholesterol (9).

The fractional absorption of cholesterol by humans (10–16) and dogs (17) has been shown to decrease when the cholesterol intake increased, while rats (18, 19) and monkeys (20) absorb the same proportion of cholesterol irrespective of dietary intake.

This report describes the effect of dietary cholesterol on absorption and excretion of cholesterol by the guinea pig. The data show that this species lacks both an effective mechanism to limit absorption of cholesterol and to respond to an increased influx with an increase of steroid excretion sufficient to maintain a stable cholesterol pool.

Certain individual guinea pigs appear resistant to the pathological effects of dietary cholesterol (hyporesponders). These hyporesponders had a decreased intestinal transit time which enabled them to absorb less cholesterol per body weight than the usual, or hyperresponding, guinea pigs.

MATERIALS AND METHODS

Animals and diets

Male Hartley guinea pigs weighing 200–250 g (Simonsen Labs., Gilroy, CA, Expts. 1 and 2; Camm Research Institute, Wayne, WV, Expts. 3 and 4) were housed individually and allowed food and water ad libitum. The control diet was Purina guinea pig chow to which had been added 5% (w/w) cottonseed oil.

¹ Present address: Dept. of Medicine, NYU Medical Center, 550 First Ave., New York, NY 10016.

² Address reprint requests to Dr. R. Ostwald, Dept. of Nutritional Sciences, 119 Morgan Hall, University of California, Berkeley, CA 94720.

Previous analysis had shown that it contained 9% (w/w) total lipid, 0.0015% (w/w) cholesterol, and 0.032% (w/w) 5- α -sterols (4). The cholesterol diets contained, in addition, 1% (w/w) or 0.1% (w/w) recrystallized (absolute ethanol) cholesterol which had been dissolved in the oil prior to mixing with the chow. Guinea pigs were adapted to the control diet for at least 2 weeks before being assigned to experimental groups.

Experimental design

In Expt. 1, cholesterol absorption by guinea pigs fed a 1% cholesterol-containing diet for 4 weeks was compared with that of guinea pigs fed the control diet. In Expt. 2, cholesterol absorption was measured in controls and 1% cholesterol-fed hyporesponders, so termed because they had not become anemic after being fed cholesterol for 44 weeks. In Expt. 3, we measured cholesterol absorption at three levels of dietary cholesterol: 0, 0.1%, and 1%, respectively.

Preliminary work had indicated that the percentage of cholesterol absorbed might increase with age. Thus, Expt. 4 was designed to measure cholesterol absorption in the same six animals at two different ages: after 6 weeks and again after 16 weeks of feeding the control diet. Because we had observed large variations in cholesterol absorption between animals within each dietary group in Expts. 1, 2, and 3, we measured absorption in each of six animals both before and after feeding them 1% cholesterol for 9 weeks (Expt. 4).

In all experiments, fractional absorption of cholesterol was measured by the fecal radioactivity method as described by Borgström (18). The fraction of cholesterol absorbed was calculated from the amount of a single dose of labeled cholesterol administered orally and the amount of radioactivity recovered in the feces. For cholesterol-fed animals, the amount of dietary cholesterol absorbed was estimated from the food intake, cholesterol content of diet, and fractional absorption of cholesterol. Recovery of non-absorbable β -sitosterol, labeled with a different isotope and administered simultaneously with the labeled cholesterol, permitted correction for possible losses of cholesterol radioactivity (10, 21).

Re-excretion of absorbed labeled cholesterol during the fecal collection period would interfere with the measurement of cholesterol absorption by the above method. In order to verify the assumption that such re-excretion of absorbed radioactivity is insignificant, we measured fecal radioactivity arising from intravenously injected [14 C]cholesterol (22). The injection of tracer cholesterol permitted measurement of excreted endogenous steroids which include

absorbed and re-excreted cholesterol, endogenously synthesized cholesterol, and bile acids. We estimated excreted steroids from the day-5 fecal radioactivity and the day-4 blood cholesterol specific activity arising from the injected tracer cholesterol (11). The amount of neutral steroids excreted was estimated similarly, using the fecal radioactivity in the neutral steroid fraction. The amount of bile acids excreted was calculated by difference.

To estimate the amount of endogenous cholesterol absorbed by control guinea pigs, we first calculated the daily endogenous secretion of steroids:

$$S = E/(1 - \%A)$$

where S is the value of secreted endogenous neutral steroids (mg/day), E is excreted endogenous neutral steroids (mg/day), and %A is the fractional absorption of cholesterol. The amount of endogenous cholesterol absorbed (A) is then: $A = (\%A)(S)$.

To obtain independent measures of cholesterol absorption, two additional methods, as described by Zilversmit (22) and Zilversmit and Hughes (23), were used. Determination of cholesterol absorption by these methods is based on the ratio of two isotopes in the blood following simultaneous oral and intravenous administration of cholesterol, each labeled with a different isotope. One method, the single measurement ratio (22), uses a single measurement of this ratio; the other, the area ratio (23), uses the ratio of the integral under the blood cholesterol specific activity curves.

The amounts of radioactivity recovered in individual animals from the orally administered [3 H]-cholesterol—the sum of absorbed and nonabsorbed (excreted) radioactivity—was better than 90% for most animals. Since the absorbed radioactivity was determined by the methods of Zilversmit (22, 23) and the excreted radioactivity by direct determination in feces, these results further validate the use of both methods of measuring cholesterol absorption.

As the purpose of this study was to determine the absorption of dietary cholesterol in the guinea pig under our usual laboratory conditions, the animals were not fasted, nor was coprophagy prevented. It has been shown that similar fractions of cholesterol were absorbed by both fed and fasted rats (24), and by rats that did and did not have access to their feces (23). Thus, it is likely that our results were unaffected by these variables.

Radioactive sterols

All sterols were obtained from Amersham/Searle, Arlington Hts., IL. [4 - 14 C]cholesterol (1–2.5 μ Ci, 140 μ Ci/mg) and [$22,23$ - 3 H] β -sitosterol (5–20 μ Ci,

113 $\mu\text{Ci}/\text{mg}$) in Expts. 1, 2, and 3; and [$1\alpha,2\alpha$ - ^3H]cholesterol (35–40 μCi , 147 $\mu\text{Ci}/\text{mg}$) alone in Expts. 4A and 4B were administered in gelatin capsules containing an accurately weighed amount (approx. 0.1 g) of cottonseed oil in which the labeled sterols had been dissolved.

For intravenous administration, colloidal [^{14}C]cholesterol (3–7 μCi) was prepared according to the method of Zilversmit (22). Immediately following the administration of the oral dose, the animals in Expt. 4 were anesthetized with Metofane (Pitman-Moore, Inc., Washington Crossing, NJ) and injected via the jugular vein. The syringe was weighed before and after the injection so that the amount of [^{14}C]cholesterol administered was accurately known.

No carrier sterols were added to the administered doses, to avoid disturbing the equilibrium established in the intestines of the guinea pigs in response to the experimental diets (10).

Radioactivity of aliquots of the oil or emulsion to be administered was measured in counting solution (toluene, 5% PPO and 0.5% bis-MSB) with a Beckman LS 230 scintillation counter (Beckman Instruments, Fullerton, CA). [^3H] or [^{14}C]toluene (Packard Instr. Co., Downers Grove, IL) was added to representative samples as internal standard, in order to correct for quenching.

Quantitation of fecal radioactivity

After administration of labeled sterols, animals were kept for 5 days in metabolic cages, which allowed separate collection of feces and urine. The daily fecal samples were collected into tared plastic bags, weighed, mixed in the bag with sufficient water to make a homogeneous paste, and frozen. The frozen samples were lyophilized, re-weighed, and pulverized. Triplicate, accurately weighed aliquots (approx. 0.1 g) were combusted in a Packard Tricarb sample oxidizer. The oxidizer was used only when better than 95% recovery of the radioactivity from ^{14}C -Speccheck and ^3H -Speccheck (Packard) was obtained. Immediately after combustion, the samples of $^{14}\text{CO}_2$ in Carbo-sorb and Permafluor V (Packard) and of $^3\text{H}_2\text{O}$ in Monophase (Packard) were counted as described above.

In Expt. 4, neutral steroids in fecal material were extracted with redistilled petroleum ether after saponification (25). The extracts were decolorized by ultraviolet light (26) and counted as described above. The radioactivity in the fecal neutral steroid fraction was similar to the total fecal radioactivity. We therefore report only the latter.

Determination of blood cholesterol and radioactivity

Blood was collected in the morning from the ear vein. Guinea pigs were not anesthetized or fasted. Sequester Sol (Ethylene Diamine Tetra Acetic Acid, Cambridge Chemical Prod., Ft. Lauderdale, FL) served as anticoagulant.

Cholesterol in whole blood was extracted with redistilled petroleum ether after saponification (27, 28), precipitated by digitonin (29) and assayed by the ferri-chloride method (30).

The radioactivity in petroleum ether aliquots was determined as described above.

Daily specific activity, dpm/mg cholesterol, was calculated for each isotope. The area under each blood specific activity curve was calculated using numerical integration; a computer program was used to fit a natural cubic spline through the data and to calculate the integral using Simpson's Rule (31).

Statistical methods

All hypothesis testing was performed by analysis of variance. For this study, the significance level was chosen to be $P = 0.05$.

RESULTS

Excretion of radioactivity from orally administered labeled sterols

Excretion of radioactivity from [^{14}C]cholesterol, [^3H]sitosterol, or [^3H]cholesterol was greatest on day 1 (Tables 1 and 2); by day 5, the amount excreted had decreased to 2%, or less, of that administered. These results demonstrate that a 5-day fecal collection was sufficient to recover all nonabsorbed radioactivity.

Absorption of labeled cholesterol with correction for nonspecific losses

The absorption of cholesterol differed in guinea pigs that responded to the 1% cholesterol-containing diet with the development of a hemolytic anemia (hyperresponders) and those that did not become anemic (hyporesponders).

In Expt. 1, the fraction excreted from the orally administered [^{14}C]cholesterol was similar for the control and 1% cholesterol-fed hyperresponding guinea pigs (Table 1). Thus, both groups absorbed a similar fraction of labeled cholesterol. Correction for losses, obtained from the recovery of ^3H from [^3H]sitosterol, did not significantly alter these results, as both

TABLE 1. Absorption of [¹⁴C]cholesterol with and without correction for nonspecific losses^a

Expt. (n)	Diet	Body Weight ^b	Iso- tope	Fecal Recovery of Dose						Cholesterol Absorption			
				1	2	3	4	5	Total	Uncor- rected ^c	Cor- rected ^c	mg/ day ^d	mg/kg/ day ^d
		g		%/ <i>day</i>					%				
1 (4)	-C	480	¹⁴ C	32 ± 10	14 ± 4	9 ± 7	2 ± 1	2 ± 0.2	58 ± 7	42 ± 7	37 ± 9	N.A.	N.A.
			³ H	55 ± 11	22 ± 6	14 ± 11	2 ± 1	1 ± 0.2	93 ± 5				
	+1%C	430	¹⁴ C	36 ± 13	20 ± 3	5 ± 0.3	2 ± 1	0.8 ± 0.1	63 ± 11	37 ± 11	34 ± 10	82 ± 22	189 ± 46
			³ H	49 ± 11	32 ± 8	9 ± 1	4 ± 1	2 ± 0.3	96 ± 4				
2 ^e (3)	-C	950	¹⁴ C	18 ± 10	13 ± 4	6 ± 1	4 ± 1	2 ± 0.4	43 ± 12	56 ± 12	55 ± 13	N.A.	N.A.
			³ H	43 ± 10	32 ± 6	11 ± 1	7 ± 4	2 ± 1	96 ± 4				
	+1%C	790	¹⁴ C	51 ± 4	11 ± 7	3 ± 1	2 ± 0.3	2 ± 0.3	69 ± 5	31 ^f ± 4	26 ^f ± 4	99 ± 13	124 ± 26
			³ H	70 ± 10	18 ± 9	4 ± 2	1 ± 0.4	1 ± 1	94 ± 4				
3 (4)	-C	660	¹⁴ C	43 ± 13	14 ± 1	4 ± 1	2 ± 0.5	2 ± 1	61 ± 10	39 ± 10	34 ± 10	N.A.	N.A.
			³ H	70 ± 10	24 ± 1	4 ± 1	1 ± 0.2	0.5 ± 0.2	94 ± 12				
	+1%C	600	¹⁴ C	25 ± 10	11 ± 2	4 ± 2	2 ± 1	1 ± 0.3	43 ± 12	57 ± 13	49 ± 15	122 ± 38	203 ± 60
			³ H	46 ± 4	25 ± 4	8 ± 4	4 ± 2	2 ± 1	85 ± 1				
+0.1%C	680	¹⁴ C	34 ± 11	15 ± 8	5 ± 2	2 ± 1	1 ± 1	52 ± 13	48 ± 13	44 ± 8	11 ^g ± 2	16 ^g ± 4	
		³ H	52 ± 17	28 ± 14	8 ± 4	2 ± 1	1 ± 0.3	91 ± 12					

^a [¹⁴C]cholesterol and [³H]sitosterol were administered as a single oral dose to groups of (n) guinea pigs fed diets containing no cholesterol (-C), 1% cholesterol (+1%C), or 0.1% cholesterol (+0.1%C). Excretion of isotope was measured by combustion of fecal samples collected on each of the 5 days following isotope administration. The +C guinea pigs in Expts. 1 and 3 had been fed cholesterol-containing diets for 4 weeks; those in Expt. 2 had been fed cholesterol for 44 weeks (hyporesponders). Values are means ± SD.

^b Mean of body weights at time of isotope administration.

$$^c \text{Uncorrected fraction of } [^{14}\text{C}]\text{cholesterol absorbed} = \left(1 - \frac{\sum ^{14}\text{C dpm excreted}}{^{14}\text{C dpm administered}} \right) \times 100.$$

$$\text{Corrected fraction of } [^{14}\text{C}]\text{cholesterol absorbed} = \left[1 - \left(\frac{\sum ^{14}\text{C dpm excreted}}{\sum ^3\text{H dpm excreted}} \right) \left(\frac{^3\text{H dpm administered}}{^{14}\text{C dpm administered}} \right) \right] \times 100.$$

^d Amounts of cholesterol absorbed were estimated from the food intake, cholesterol content of diet, and corrected fractional absorption. N.A.—not analyzed.

^e Rate of excretion of either labeled sterol by +1%C guinea pigs was greater than that of -C guinea pigs as determined by a two-factor analysis of variance, $P < 0.001$.

^f +1%C vs. -C groups statistically different at $P < 0.05$.

^g +1%C vs. +0.1%C groups different at $P < 0.001$.

groups excreted more than 90% of the labeled sitosterol (Table 1).

The hyporesponding 1% cholesterol-fed guinea pigs excreted more, and thus absorbed less, [¹⁴C]-

cholesterol radioactivity than did their controls (Table 1: Expt. 2). Again, the percentage of cholesterol absorbed, when corrected for losses, did not differ statistically from the uncorrected values, as more than

90% of the administered [^3H]sitosterol was excreted by both groups.

The hyporesponders had a shorter intestinal transit time than either their controls or similar-sized 1% cholesterol-fed hyperresponders. On the first day, they excreted 70% of the nonabsorbable sterol compared to 43% for the controls (Table 1: Expt. 2), and they excreted more than twice as much labeled cholesterol as did the controls or similar-sized hyperresponders (Table 2: Expt. 4B). Consequently, the amount of cholesterol absorbed by the hyporesponders was 65–86 mg/kg per day less than that absorbed by the hyperresponders in Expts. 1, 3, and 4B (Tables 1 and 3).

The results of Expt. 3 (Table 1) show that there was no statistically significant increase of the fractional absorption of cholesterol with increasing dietary cholesterol. The amount of cholesterol absorbed by the animals fed 1% cholesterol was, however, 10 times greater than that absorbed by animals fed 0.1% cholesterol.

Relationship of cholesterol absorption and aging

The relationship of cholesterol absorption and aging was of interest as the animals fed a control diet for 44 weeks (Expt. 2) absorbed a somewhat larger proportion of the labeled cholesterol than did the animals of Expts. 1 and 3 fed the same diet for 4 weeks. Cholesterol absorption, however, did not significantly increase in animals fed the control diet for 6 or 16 weeks (Table 2: –C, Expts. 4A and 4B). The fraction of cholesterol absorbed by these animals was intermediate between that of the old animals of Expt. 2 and of the young animals of Expts. 1 and 3. Either the time between the two parts of Expt. 4 was

not long enough, or the animals were not young enough at the first time point to observe an unambiguous effect of aging on the absorption of cholesterol.

Excretion of injected [^{14}C]cholesterol

Re-excretion of absorbed labeled cholesterol, over the 5 days of fecal collection, was insignificant for the purpose of this experiment. This follows from the observation that at the end of 5 days the total fraction of an injected dose excreted by the control and 1% cholesterol-fed guinea pigs was only $11 \pm 3\%$ and $8 \pm 2\%$, respectively. All animals excreted similar proportions of the injected dose on days 1, 2, and 3. On days 4 and 5, however, the 1% cholesterol-fed guinea pigs excreted a smaller proportion of the dose than did their controls (1.7 ± 0.8 and 2.9 ± 0.8 for day 4; 1.5 ± 0.3 and 2.8 ± 1.1 , for day 5, respectively; $P < 0.05$). The interpretation of this finding will be discussed later.

Absorption of [^3H]cholesterol by guinea pigs before and after inclusion of cholesterol in the diet

The fraction of labeled cholesterol absorbed was not affected by dietary cholesterol as shown by measurement of absorption in the same group of animals before and after feeding the 1% cholesterol-containing diet (Table 2: Expt. 4A vs. 4B). This confirms our results from Expts. 1 and 3 where absorption was compared between different groups of guinea pigs fed either the control diet or the 1% cholesterol-containing diet. By measuring absorption in the same animals before and after cholesterol feeding, we have minimized the variability within each group; thus, any changes in the fractional absorption in response

TABLE 2. Effects of dietary cholesterol and aging on fractional absorption of [^3H]cholesterol^a

Expt.	(n)	Diet	Body Weight ^b	Fecal Recovery of Dose					Total	[^3H]Cholesterol Absorbed ^c
				1	2	3	4	5		
			g	% / day					%	
4A	(6)	–C ^d	580	22 ± 10	15 ± 6	7 ± 2	3 ± 2	2 ± 2	49 ± 13	51 ± 13
		–C ^e	590	26 ± 9	13 ± 2	7 ± 4	3 ± 2	2 ± 1	51 ± 6	48 ± 6
4B	(6)	–C ^d	927	22 ± 7	13 ± 4	6 ± 1	4 ± 2	2 ± 1	46 ± 7	54 ± 7
		+1% C ^e	750	18 ± 11	13 ± 5	8 ± 5	3 ± 2	1 ± 0.4	44 ± 13	56 ± 13

^a Excretion of isotope from [^3H]cholesterol, administered as a single oral dose, was determined before and after 9 weeks of feeding 1% cholesterol (+1% C) to six guinea pigs, and at two time points (6 and 16 weeks) in six guinea pigs fed a cholesterol-free diet (–C). The total radioactivity excreted was measured by combustion of fecal samples collected on each of the 5 days following administration. Values are mean ± SD.

^b Mean of body weight at time of isotope administration.

^c Fractional absorption of [^3H]cholesterol = $\left(1 - \frac{\sum ^3\text{H dpm excreted}}{^3\text{H dpm administered}}\right) \times 100$. Amounts of cholesterol absorbed by these guinea pigs are shown in Table 3.

^d Same animals.

^e Same animals.

to the diet should have become more apparent. As there were no changes, we must conclude that guinea pigs do not adapt to a 1% cholesterol-containing diet by decreasing the fractional absorption of cholesterol.

Steroid balance

The data in Table 3 show that the excretion of both total and neutral steroids, which include endogenous steroids and absorbed dietary cholesterol, was greatly increased in the cholesterol-fed guinea pigs compared to the controls. The data also show that the amount of cholesterol absorbed by these guinea pigs was three times greater than the total amount of steroids excreted. This difference between input and output is a measure of the net increase of cholesterol pool. In contrast, excretion of steroids by the control guinea pigs greatly exceeded the re-absorption of endogenous cholesterol.

Cholesterol absorption as measured by the Zilversmit methods

Table 4 shows that, for all groups, the ratio of the orally to the intravenously administered cholesterol in the blood was lower on day 1 than on succeeding days. This finding has also been reported for the rat (23). The ratio on day 3 was arbitrarily chosen to represent cholesterol absorption (22). The fraction of cholesterol absorbed by the controls and 1% cholesterol-fed guinea pigs was similar. When we used the area ratio method (23) for the evaluation of the data, the results were not statistically different from those obtained by the single measurement ratio (Table 4). The results are also similar to those ob-

TABLE 3. Steroid balance: absorption and excretion

Diet	(n)	Excreted ^a		Absorbed ^b	
		Total Steroids	Neutral Steroids	Cholesterol	
		mg/day	mg/day	mg/day	mg/kg/day
-C	(5)	14 ± 8 ^c	1.6 ± 0.4 ^c	1.7 ± 0.5 ^d	2 ± 1 ^d
+1%C	(5)	52 ± 11	4.9 ± 1.5	160 ± 30	210 ± 40

^a The amount of total steroids excreted (bile acids plus neutral steroids) was estimated from the day-4 blood cholesterol specific activity (dpm/mg) and the day-5 total fecal radioactivity following intravenous administration of [¹⁴C]cholesterol (11). The amount of neutral steroids excreted was calculated similarly using the fecal neutral steroid radioactivity. The animals are from Expt. 4B. They had been fed for 16 weeks a cholesterol-free diet (-C) or for 9 weeks a 1% cholesterol-containing diet (+1% C). Data of only 5 animals are included because of technical problems encountered with one animal of each group. Values are mean ± SD.

^b The amount of endogenous cholesterol absorbed by animals fed a cholesterol-free diet was estimated from the amount of neutral steroids secreted (see Methods) and the fractional absorption of cholesterol. The amount of cholesterol absorbed by the 1% cholesterol-fed guinea pigs was estimated from their food intake, cholesterol content of the diet, and the fractional absorption of cholesterol.

^c +1%C vs. -C groups statistically different at $P < 0.05$.

^d +1%C vs. -C groups statistically different at $P < 0.001$.

tained by the fecal radioactivity method for the same animals (Table 2).

DISCUSSION

One of the postulated mechanisms to control cholesterol pool size is the limitation to the absorption of dietary cholesterol (7). We have shown that the guinea pig does not utilize this mechanism. The same

TABLE 4. ³H/¹⁴C ratio in blood after simultaneous oral administration of [³H]cholesterol and intravenous administration of [¹⁴C]cholesterol^a

Expt. (n)	Diet	Single Measurement Ratio ^b ³ H/ ¹⁴ C					Area Ratio ^c $\frac{\int ^3\text{H S.A.}}{\int ^{14}\text{C S.A.}}$
		1	2	3	4	5	
4A (4)	-C ^d	31 ± 7	40 ± 6	43 ± 7	44 ± 8	44 ± 7	40 ± 7
	-C ^e		51 ± 31	52 ± 30	51 ± 29	47 ± 25	
4B (5)	-C ^d	35 ± 15	42 ± 16	45 ± 16	46 ± 16	46 ± 16	47 ± 16
	+1%C ^e	33 ± 9	49 ± 23	49 ± 23	49 ± 23	48 ± 22	

^a Ratios were adjusted to equal radioactivity from each isotope and therefore represent cholesterol absorption. Guinea pigs were tested before and after feeding a 1% cholesterol-containing diet for 9 weeks (+1% C) and at two time points of feeding a cholesterol-free diet (6 and 16 weeks) (-C). Data are reported for only those animals that had received a quantifiable injection of tracer.

^b Ratio of ³H and ¹⁴C for days 1 to 5.

^c Ratio of the integrals of the blood cholesterol specific activity (S.A.) curves. Half of the animals of Expt. 4B were autopsied, so there were only three animals/group in Expt. 4B for the measurement of area ratio.

^d Same animals.

^e Same animals.

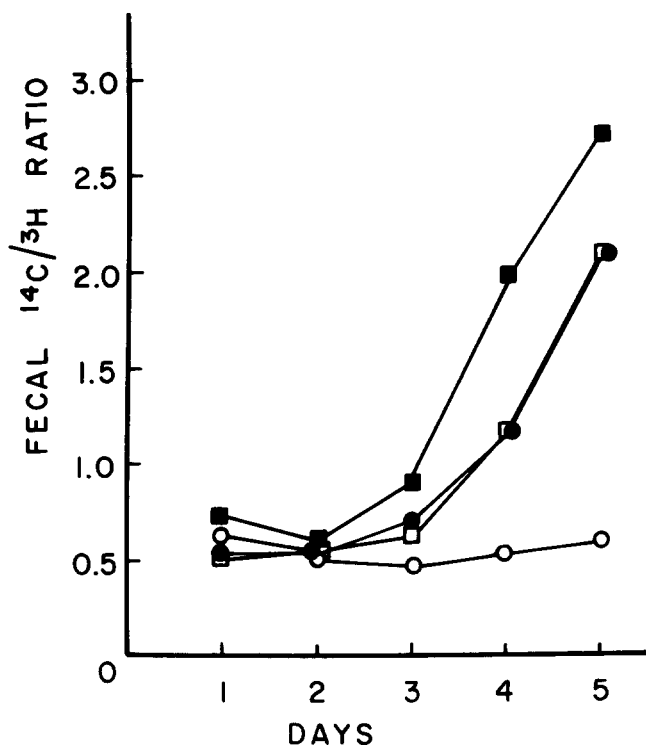


Fig. 1. Daily fecal $^{14}\text{C}/^3\text{H}$ ratios following oral administration of [^{14}C]cholesterol and [^3H]sitosterol. ^{14}C and ^3H were measured in five daily fecal samples from guinea pigs fed a cholesterol-free diet (-C, ●), a diet containing 0.1% cholesterol (+0.1% C, □), or a diet containing 1.0% cholesterol (+1% C, ○; and +1% C hyperresponders, ■). The $^{14}\text{C}/^3\text{H}$ ratios have been adjusted to equal radioactivity from each isotope and therefore should represent the fraction of cholesterol absorbed. The data shown are means for -C guinea pigs from Expts. 1, 2, and 3; +1% C guinea pigs from Expts. 1 and 3; hyporesponding +1% C guinea pigs from Expt. 2; and 0.1% C guinea pigs from Expt. 3 (Table 1). The ratios of isotopes administered varied from 5.1 to 6.8 in the different experiments. By two-factor analysis of variance, the -C vs. +1% C curves for both Expts. 1 and 3 were significantly different ($P < 0.001$).

fraction of tracer cholesterol was absorbed whether the diet contained 0, 0.1%, or 1% cholesterol (Table 1). Furthermore, there was no change in the proportion of tracer cholesterol absorbed by the same animals when the cholesterol-free diet was changed to one containing 1% cholesterol (Table 2). Since the fraction of cholesterol absorbed was constant over the range of dietary cholesterol levels studied, the amount of cholesterol absorbed increased in proportion to the amount of cholesterol consumed.

The hyporesponding 1% cholesterol-fed guinea pigs are an exception to this finding. These animals were studied because they had been fed a diet containing 1% cholesterol for nearly a year without suffering from the anemia that occurs in most guinea pigs fed this diet; however, their plasma cholesterol did

increase somewhat above control levels.³ These hyporesponding guinea pigs absorbed a smaller fraction of ingested tracer cholesterol than did similar-aged controls (Table 1: Expt. 2) and similar-sized hyperresponders (Table 2: Expt. 4B). Buchwald and Gebhard (32) have shown that the proportion of cholesterol absorbed is dependent on the intestinal transit time. Since intestinal transit time was shorter in the hyporesponding than in the hyperresponding guinea pigs (Table 1), we suggest that this phenomenon resulted in the lower fractional absorption. As less cholesterol was absorbed by the hyporesponders than by hyperresponders (per unit of body weight), this may account for the decreased pathological effects of exogenous cholesterol in the hyporesponders. Other possible mechanisms such as an increase in steroid excretion, decrease of cholesterol synthesis, or sequestration of excess cholesterol in the liver have not been investigated in the present study.

A similar phenomenon has been studied in the rhesus monkey (33). Monkeys were compared on the basis of their serum cholesterol in response to an atherogenic diet. The low-responders had a significantly lower fractional absorption of cholesterol than did the high-responders. No mechanism for this difference was suggested.

Guinea pigs fed 0.1% cholesterol also do not become hypercholesterolemic or anemic (4). Unlike the hyporesponding 1% cholesterol-fed guinea pigs, these guinea pigs absorbed the same fraction of tracer cholesterol as did their controls (Table 1: Expt. 3). The amount absorbed, however, was small enough to permit these animals maintenance of cholesterol homeostasis by a combination of increased excretion and of sequestration of excess cholesterol in the liver (4).

It has been suggested that cholesterol absorption can be estimated from the ratio of fecal radioactivities derived from a single orally-administered dose containing cholesterol and nonabsorbable sitosterol, each sterol labeled with a different tracer (34). It has been found, however, that this ratio does not always remain constant with time. In humans (10, 13, 35) and in rabbits (35), the ratio in successive samples increased, while it remained unchanged in a patient with obstructive liver disease (10) and in rats with bile fistulae (35). In the last two cases, insufficient bile flow prevented cholesterol absorption. We have found that the ratio of fecal cholesterol/sitosterol radioactivities increased on days 4 and 5 for all groups, except for the hyperresponding 1% cholesterol-fed

³ Traber, M. G. and R. Ostwald. Unpublished observations.

guinea pigs, in which the ratio remained constant (Fig. 1). As the hyperresponders do absorb cholesterol, lack of absorption cannot explain this phenomenon.

One hypothesis to explain the increasing ratio proposes that biliary (or re-excreted) tracer cholesterol becomes a progressively larger fraction of the fecal radioactivity as compared to the nonabsorbed tracer sitosterol (35). Our results support this hypothesis. As we pointed out above, control animals excreted an increasing fraction of the injected tracer cholesterol on days 4 and 5, while the fraction excreted by the 1% cholesterol-fed guinea pigs decreased. If the pattern for re-excretion of absorbed tracer cholesterol is similar to that found for injected tracer cholesterol, it would cause the changes in the fecal $^{14}\text{C}/^3\text{H}$ ratio that we found. Since we did not feed labeled sitosterol in Expt. 4, we cannot validate this hypothesis directly. Mucosal cells of 1% cholesterol-fed hyperresponders, but not those of the other groups, would be expected to be loaded with [^{14}C]-cholesterol. Sloughing of these cells might therefore contribute to the unchanging ratio of fecal $^{14}\text{C}/^3\text{H}$ observed in this group.

The data for steroid excretion reported here are approximations. Guinea pigs fed a 1% cholesterol-containing diet are not in steady state, so cholesterol turnover studies are not possible. Furthermore, as guinea pigs lick their fur and practice coprophagy, steroids from these sources are introduced into the gut. Thus, chemical analysis of gut contents for steroids does not yield an accurate measure of steroid excretion. Furthermore, our estimation assumes that the specific activity of the blood cholesterol is the same as the specific activity of the steroids secreted into the gut. We have shown that the transit time is approximately one day, since between 50 and 75% of the absorbable sterol was excreted within 24 hr. Thus our method to estimate steroid excretion using the total fecal radioactivity on day 5 and the blood specific activity on day 4 would seem to be justified. The values for the excretion of steroids by guinea pigs obtained by this method (18 mg/kg per day) are similar to those reported previously from input-output analysis (25 mg/kg per day) (4). These measurements are therefore adequate for the purpose of comparisons of cholesterol absorption and excretion.

Excretion of bile acids and of neutral steroids is an important mechanism in the control of cholesterol pool size. Rats, when fed a cholesterol-containing diet, are able to sufficiently increase bile acid excretion to offset increased absorption (36, 37). Humans

and dogs, in contrast, limit cholesterol pool expansion by a combination of decreased absorption and an increase in the excretion of neutral steroids (16, 17). Our data show that guinea pigs increase excretion of both bile acids and neutral steroids by a factor of 3 (Table 3) (8). They can, however, maintain cholesterol pool size only if the influx of cholesterol is relatively small, since they cannot control its absorption. The 1% cholesterol-fed guinea pigs absorbed 100 mg cholesterol/day more than they excreted in any form. Furthermore, they absorbed ten times as much cholesterol as the 0.1% cholesterol-fed guinea pig, but excreted only twice as much (69 and 37 mg/kg per day for the 1% and 0.1% cholesterol-fed guinea pigs, respectively) (Table 3) (4). ■■

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