

Quantification of cholesterol absorption in man by fecal analysis after the feeding of a single isotope-labeled meal

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ABSTRACT The fecal excretion of cholesterol-4-¹⁴C and β -sitosterol-22,23-³H has been studied in normal human subjects after they had ingested a single meal containing the radioactive substances.

When 150 mg of β -sitosterol, dispersed in the butter of a standard breakfast, was fed to 20 subjects the mean recovery of isotope in the feces was 90%. When plant sterols (70% β -sitosterol, 30% campesterol) were fed together with cholesterol and used as an internal standard to correct for losses of cholesterol during intestinal transit and analytical procedures, excretion of dietary cholesterol was found to be 60–80%, irrespective of the amount fed over the range 150–1910 mg. If absorption of cholesterol is calculated from these figures, no saturation of the cholesterol absorption mechanism is indicated for the amounts of cholesterol fed in this investigation.

The reason for the differences between these findings and those previously reported by other procedures is not clear, but may be related to the acute administration of a single dose of cholesterol in this study.

SUPPLEMENTARY KEY WORDS intestinal absorption · external and internal standards · β -sitosterol · fecal excretion

THE QUANTITATIVE aspects of intestinal absorption of cholesterol in the human have attracted considerable interest in recent years. Calculations from data obtained in isotope experiments either in the building-up phase (1) or in the steady state (2) have given results which indicate that absorption of dietary cholesterol in the human is limited by a ceiling and does not exceed 200–300 mg/day even when large doses of cholesterol are fed. These figures are in contrast to the much higher values of 2–3 g/day obtained from nonisotopic balance

studies (3). Calculation of absorption of dietary cholesterol from both these types of experiments, however, involves certain assumptions, the validity of which can affect the results considerably. The calculations from the isotope experiments are based on the size of the replaceable cholesterol pool (1) or the turnover rate of cholesterol (2), parameters that are only approximately known. The nonisotopic balance studies are complicated by the admixing of endogenous cholesterol in the intestinal tract in quantities that are significant in relation to the daily intake of cholesterol.

More information therefore seemed desirable about the quantitative aspects of cholesterol absorption in the human, and the present experiments were undertaken to explore what information could be derived from fecal analysis after a single feeding of meals containing labeled cholesterol. Because it has been reported that the plant sterol β -sitosterol is absorbed from the human intestine only in negligible amounts (4), it was also of interest to relate the relative excretion figures of these two sterols when they were fed together.

METHODS

Volunteers of both sexes between 16 and 45 yr old were used. After an overnight fast they consumed a breakfast of coffee, bread, and marmalade with 50 g of butter as the only visible source of fat. They received no dietary pretreatment, and were on their normal diets for the fecal collection period. The butter contained the sterols indicated in the tables and figures. The cholesterol content of the butter was determined in several butter specimens; a mean figure of 0.3% (w/w) was used for the calculation of the inherent cholesterol con-

tent of the butter fed. The sterols were added to butter melted in a water bath, and the mixture was stirred continuously until the sterol was dissolved. The butter was then cooled in ice water with continued stirring until a homogeneous butter with normal consistency was obtained. The butter was then dispensed into plastic containers, each containing 50 g, and stored at 4°C until used.

In a first series of experiments on 28 experimental subjects, cholesterol in doses from 150 mg to 1.91 g/50 g of butter was fed. Each 50 g of butter contained 1 μ C of cholesterol-4-¹⁴C. In this series, β -sitosterol-22,23-³H (see below) was added as external standard to the fecal samples before extraction.

In a second series of experiments on 24 subjects, the butter contained both cholesterol-¹⁴C and β -sitosterol-³H in the amounts given in the tables and figures. In most of these experiments 150 mg of sitosterol was used per 50 g of butter, the cholesterol content being between 150 mg and 1.91 g. Four subjects were given 950 mg of β -sitosterol-³H with 950 mg of cholesterol-¹⁴C.

Cholesterol-4-¹⁴C was obtained from the Radiochemical Centre, Amersham, England. Each batch was tested for radiopurity by thin-layer chromatography. If the purity was less than 99% the sample was purified by thin-layer chromatography on 1 mm-thick silica plates.

Cholesterol was a product of Eastman-Kodak Co. and was recrystallized from acetone. β -Sitosterol-22,23-³H was prepared as described earlier (5). Unlabeled " β -sitosterol" was obtained from Sigma Chemical Co. and contained campesterol and β -sitosterol in the proportion 3:7; in view of its impurity, we refer to it here as "plant sterols."

Fecal Analysis

In most cases feces were collected daily for 5–6 days after feeding and the daily collections were separately analyzed. In some cases the feces from 5 days were pooled and analyzed as such.

Two methods of extraction were used.

Extraction with Chloroform–Methanol 1:1. Feces were collected in methanol (300 ml for daily specimens) and homogenized by means of a motor-driven propeller. 500 ml of chloroform and 200 ml of methanol were added, and the homogeneous mixture was stirred for 20 min. The sample was left overnight to sediment; the clear supernatant solution was filtered through ordinary filter paper and a 100 ml aliquot of the filtrate was removed to a separatory funnel. After addition of 40 ml of water the chloroform phase separated and 50 ml of it was taken for further analysis (= 1/10 of the total chloroform phase). For the 5-day fecal pools, the analysis

proceeded in the same way except that all the volumes were five times as large.

The chloroform aliquots were evaporated to dryness and the lipids were saponified overnight. The neutral lipids were extracted with light petroleum from the alkaline lower phase, which contained 50% ethanol in water. The nonsaponifiable material extracted was always strongly colored and was decolorized by ozonization (6) before its radioactivity was determined.

Extraction According to Grundy, Ahrens, and Miettinen (7). The feces were homogenized in an equal weight of water and a 10 ml portion was weighed and saponified. The neutral lipids extracted were decolorized as described above and their radioactivity was determined. In some experiments the two methods of extraction were compared. In these experiments the feces were homogenized with water 1:1 and one portion was used according to the method of Grundy et al. (7) while another aliquot of 10 ml was extracted with chloroform–methanol as described above. Table 1 shows that the methods give similar results.

Radioactivity was measured by liquid scintillation counting in a Packard spectrometer. Decolorization by ozonization led to samples with a very low degree of quenching. Internal standardization was generally used to evaluate quenching in the samples. The correction factor was usually found to be lower than 1.10 and with no significant difference for ¹⁴C and ³H. Because of the errors inherent in the addition of the internal standard, the results have not been corrected for quenching.

TABLE 1 RADIOACTIVITIES RECOVERED FROM FECES OF SUBJECTS FED β -SITOSTEROL-22,23-³H AND CHOLESTEROL-4-¹⁴C

Day	Subject			
	CS		AN	
	A	B	A	B
	<i>% of radioactivity fed</i>			
¹⁴ C				
1	0.4	0.5	0.1	0.2
2	52.1	49.1	21.3	21.6
3	14.8	15.1	28.8	24.8
4	3.2	2.6	5.9	5.9
5	0.6	0.4		
Sum:	71.1	67.7	56.1	52.2
³ H				
1	0.8	1.1	0.1	1.9
2	71.8	70.0	36.7	36.6
3	17.7	17.8	37.9	38.2
4	3.0	3.4	5.0	5.5
5	0.6	1.0		
Sum:	93.9	93.3	79.6	80.3

Two different extraction procedures were used. A, the procedure described in this paper utilizing extraction with chloroform–methanol. B, the procedure described by Grundy, Ahrens, and Miettinen (7).

TABLE 2 FECAL EXCRETION OF RADIOACTIVITY AFTER FEEDING HUMANS 50 g OF BUTTER CONTAINING CHOLESTEROL-4-¹⁴C AND β-SITOSTEROL-22,23-³H

Isotope Measured	% Excretion of Isotopes on Day						Absorption of Cholesterol		
	1	2	3	4	5	6	1-6	Uncorrected	Corrected*
<i>150 mg cholesterol; 150 mg β-sitosterol</i>									
¹⁴ C	1.2	18.8	20.5	4.2	1.3	—	46.0	54.0	49.9
³ H	0.3	44.3	42.9	4.1	0.6	—	92.2	7.8	—
¹⁴ C	11.2	11.0	15.5	8.7	12.1	3.8	62.3	37.7	34.7
³ H	20.0	18.5	24.5	13.1	15.6	3.9	96.6	4.4	—
¹⁴ C	6.8	41.5	3.1	1.3	0.3	—	53.0	47.0	37.2
³ H	14.5	67.1	2.2	0.5	0.1	—	84.4	15.6	—
¹⁴ C	32.3	22.1	3.5	0.3	—	—	58.2	41.8	36.7
³ H	54.9	33.3	3.6	0.2	—	—	92.0	8.1	—
¹⁴ C	—	—	—	—	—	—	57.1	42.9	32.6
³ H	—	—	—	—	—	—	84.9	15.1	—
¹⁴ C	—	—	—	—	—	—	49.0	51.0	46.4
³ H	—	—	—	—	—	—	91.5	8.5	—
Mean:							¹⁴ C	53.0	45.7
							³ H	77.4	9.9
<i>550 mg cholesterol; 150 mg β-sitosterol</i>									
¹⁴ C	4.8	20.0	4.4	0.7	0.4	—	30.3	69.7	25.6
³ H	7.9	29.4	3.0	0.2	0.2	—	40.7	59.3	—
¹⁴ C	5.2	26.5	—	3.5	2.2	—	37.4	62.6	66.0†
³ H	8.5	97.8	—	3.2	0.8	—	110.3	-10.3	—
¹⁴ C	0.4	52.1	14.8	3.2	0.6	—	71.1	28.9	24.2
³ H	0.8	71.8	17.7	3.0	0.6	—	93.9	6.1	—
¹⁴ C	0.1	21.3	28.8	—	5.9	—	56.1	43.9	29.6
³ H	0.1	36.7	37.9	—	5.0	—	79.7	20.3	—
¹⁴ C	0.2	30.3	17.0	10.0	1.2	—	58.6	41.4	35.0
³ H	0.4	46.8	28.8	13.9	0.3	—	90.2	9.8	—
¹⁴ C	13.9	32.2	8.7	0.3	0.7	—	55.8	44.2	36.9
³ H	25.4	53.0	9.5	0.3	0.3	—	88.5	11.5	—
Mean:							¹⁴ C	51.5	48.5
							³ H	83.9	16.1
<i>950 mg cholesterol; 150 mg β-sitosterol</i>									
¹⁴ C	—	29.9	21.8	4.0	3.3	—	59.0	41.0	27.5
³ H	—	43.5	29.5	5.1	3.2	—	81.3	18.7	—
¹⁴ C	0.1	0	1.9	20.1	29.4	9.8	61.3	38.7	21.2
³ H	0.1	0.1	2.6	25.3	37.5	12.1	77.7	20.3	—
¹⁴ C	27.7	35.6	2.1	0	—	—	65.4	34.6	30.2
³ H	45.6	47.7	0.4	0	—	—	93.7	6.3	—
¹⁴ C	0.3	30.5	45.9	3.0	1.5	—	81.2	18.8	17.2
³ H	0.1	38.6	55.2	3.2	0.9	—	98.0	2.0	—
Mean:							¹⁴ C	66.7	33.3
							³ H	88.2	11.8
<i>1910 mg cholesterol; 150 mg β-sitosterol</i>									
¹⁴ C	3.0	42.5	24.1	4.0	0.5	—	74.1	25.9	24.0
³ H	5.2	54.8	31.3	5.0	1.4	—	97.7	2.3	—
¹⁴ C	0.4	52.8	19.4	6.3	0.9	—	79.8	20.2	26.0
³ H	6.6	67.1	24.7	5.3	4.6	—	108.3	-8.3	—
¹⁴ C	37.8	28.5	11.1	—	1.3	—	78.7	21.3	19.3
³ H	47.8	36.1	11.7	—	1.8	—	97.4	2.6	—
¹⁴ C	—	17.6	—	50.2	—	—	8.3	76.1	23.9
³ H	—	26.5	—	66.7	—	—	5.6	98.8	1.2
Mean:							¹⁴ C	77.2	22.8
							³ H	100.6	-0.6
<i>950 mg cholesterol; 950 mg β-sitosterol</i>									
¹⁴ C	11.6	35.0	22.2	4.2	0.6	—	73.6	26.4	17.7
³ H	14.5	43.3	26.3	5.0	0.4	—	89.5	10.5	—
¹⁴ C	20.4	44.7	15.0	—	2.2	—	82.3	17.7	18.4
³ H	28.9	52.4	17.2	—	2.5	—	101.0	-1.0	—
¹⁴ C	45.2	20.5	—	10.7	3.3	—	79.7	20.3	16.8
³ H	55.5	25.7	—	11.1	3.5	—	95.8	4.2	—
¹⁴ C	—	14.2	30.4	8.1	7.9	—	60.5	39.4	21.0
³ H	—	17.8	39.0	10.6	9.2	—	76.6	23.4	—
Mean:							¹⁴ C	74.0	26.0
							³ H	90.7	9.3

* Corrected to a theoretical 100% β-sitosterol excretion.

† This figure has been excluded from the mean given in parentheses.

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

RESULTS

In the first series of experiments cholesterol-4- ^{14}C was fed to 25 subjects in amounts ranging from 150 mg to 1.91 g in 50 g of butter in a single meal. Feces were collected for 5 days and β -sitosterol- ^3H was added to the feces (dispersed in methanol) as an external standard to correct for losses during the extraction procedure. The mean recovery of externally added β -sitosterol activity was 96.0%. For cholesterol the fecal excretion, corrected for 100% β -sitosterol recovery, was 64.5 (7), 68.7 (4), 66.4 (4), and 70.4% (7) for 150, 550, 950, and 1910 mg fed in a single meal (the number of subjects in each group in parentheses). If one of the subjects fed 1910 mg, who showed an excretion of only 32.8%, were to be ignored the figure for this group would be 76.7%.

To three subjects we fed 50 g of butter with 1.91 g of labeled cholesterol three times in 1 day (= 5.73 g) and collected the feces for 5 days. The fecal recoveries in these experiments gave a mean excretion of 73.4% (corrected for external β -sitosterol), a figure almost identical with the one obtained for the subject fed one meal with 1.91 g of cholesterol.

Table 2 shows values in the second series of experiments, in which the subjects were fed plant sterols containing β -sitosterol-22,23- ^3H with different doses of cholesterol-4- ^{14}C . The excretion pattern varies from subject to subject; in some the largest fraction of the fed sterols was already excreted on the 1st day after the feeding, whereas a few cases show a very slow passage through the intestinal tract, with excretion of the isotope delayed to the 4th and even the 5th day. In only 4 out of 24 cases, however, did the last fecal sample analyzed contain more than 5% of the dose fed.

The mean fecal excretion figure for plant sterol in the 20 subjects fed 150 mg of labeled β -sitosterol was 89.9%. If one exceptional figure showing 40% excretion is excluded from the data the mean becomes 92.5%. In the series with four subjects fed 950 mg of cholesterol and 950 mg of β -sitosterol the mean excretion of sitosterol was 90.7%.

The mean cholesterol excretion percentages directly measured in this series were 53.0 (6), 51.5 (6), 66.7 (4), and 77.2% (4) for 150, 550, 950, and 1910 mg of cholesterol fed. When these figures are corrected to a theoretical 100% sitosterol excretion, the corresponding figures are 60.4, 63.8, 76.0, and 76.8%. These figures are not significantly different ($P > 0.05$) from those obtained in the first series (in which an external sitosterol standard was used) except for the 950 mg dose.

When 950 mg of cholesterol was fed with 950 mg of β -sitosterol, the cholesterol recovery was 81.5% compared to 76.0% when it was fed with 150 mg of sitosterol. This difference is not statistically significant.

DISCUSSION

The results of the present investigation have been given so far in the form of fecal excretion of dietary sterols. Under certain conditions the assumption can be made that the radioactive compound not excreted is absorbed from the intestinal tract. The criteria to be fulfilled if excretion is to be directly related to absorption can be listed as follows.

(a) The method used for recovery of isotope from feces should be adequate.

(b) The collection period of stools should be long enough compared to the transit time through the intestinal tract.

(c) Absorbed isotope should not be reexcreted to the intestinal tract in appreciable amounts.

(d) Exchange of radioactive with nonradioactive material between the intestinal content and the intestinal wall should not take place.

(e) The radioactive material should not be degraded in the intestinal tract to a chemical form that is not recovered by the method of analysis.

(f) The radioactive compound fed should not become mixed in the intestinal tract with comparable quantities of nonradioactive material from endogenous sources.

The first two criteria seem to be satisfied in the present experiments. The recoveries of isotope from the feces with the method used are similar (Table 1) to those of others (7). In most of the experiments daily fecal samples were collected and analyzed, and in most of the cases the major proportion of radioactivity excreted appeared in the 2nd and 3rd day collections, with only minor amounts excreted later. The results do show, however, that the individual variations in transit time through the intestinal tract are important and have to be considered. Any reexcretion of absorbed isotope to the intestine does not seem to be important, as the excretion rapidly tapers off during the later portion of the collection period. Experiments in the rat indicate that, in this species at least, less than 10% of absorbed cholesterol is reexcreted to the intestinal tract (8). Before being reexcreted to the intestinal tract, any absorbed cholesterol can be expected to equilibrate with the plasma-red cell-liver pool, the size of which is around 16 g in man (1).

Exchange of labeled cholesterol in the intestinal tract with unlabeled cholesterol (i.e., the replacement of an unlabeled cholesterol molecule in mucosal cells by a labeled one, with resultant transfer of label from lumen

to mucosal cell without net movement of cholesterol) does not seem to occur in the rat (9); it has not been definitely demonstrated in the human, mainly because of methodological difficulties (10). However, this phenomenon has not been ruled out, particularly in man, and cholesterol absorption measured by both present and previously described techniques (1, 2) may be overestimated.

Until recently it has been assumed that the sterol nucleus was not degraded in the intestinal tract. Experiments by Grundy, Ahrens, and Salen (11) have, however, definitely demonstrated that large quantities of sterols can disappear from the intestinal tract of man, presumably by bacterial degradation.

Because sitosterol absorption from the intestine is negligibly small (4), the above authors have suggested that sitosterol can be used as an internal standard for dietary cholesterol if one assumes that any loss of sitosterol in the intestinal tract is accompanied by the same percentage loss of cholesterol.

Considerable quantities of endogenous cholesterol pass into the intestinal tract via the bile and possibly through the intestinal mucosa (10). The quantities of sterol secreted to the human intestine have been calculated to be 1–2 g/day (12, 13).

We conclude that the results of single-dose isotopic balance studies with sterols will give a fair expression of the amount of radioactive material absorbed, the danger being that some of it will be degraded in the intestinal tract and give too high figures for absorption in some individuals. On the other hand, the figures obtained from isotopic data will not represent the total mass of cholesterol absorbed from the intestinal tract; this will be (because of the addition of endogenous cholesterol to the intestinal tract) considerably larger.

In the following discussion it is assumed that the radioactive sterol not excreted is absorbed, and the data are presented in terms of sterol absorption.

β -Sitosterol Absorption

The intestinal absorption of sitosterol in man was calculated by Gould, Lotz, and Lilly (4) by an indirect method to be about 4% of a single dose fed. The fecal recoveries in the steady state after feeding plant sterol to humans have indicated an excretion of β -sitosterol of up to 95% in the experiments of Grundy et al. (11). The figures obtained for absorption of β -sitosterol in this investigation for the 150-mg dose fed were, as a mean of 19 experiments, $7.5 \pm 1.9\%$. Excluded from this mean are the results from one experimental subject who excreted only 40% of the sitosterol fed and thus had an apparent absorption of 60%. In this case no obvious reason for the low recoveries could be found; most of the isotope was excreted during the first 2 days of collection, and the

experimental subject was considered reliable. Probably, therefore he has to be referred to the category of normal human beings in whom the sterol nucleus is degraded in the intestinal tract. This subject also showed a comparatively low excretion of labeled cholesterol. Among the remaining 19 experimental subjects fed 150 mg of plant sterols, 5 showed an apparent absorption that differed from the mean by more than three times the standard deviation. In these cases also a bacterial degradation may be involved, even if in some of them a slow transit with incomplete collection could be an additional factor. The mean figure of 10.1% obtained for β -sitosterol absorption in this investigation is, therefore, probably too high, and the indirect figure obtained by Gould et al. is closer to the real absorption. Indeed, in all studies in which sitosterol absorption is based on fecal recovery after oral administration, the absorption will be overestimated to the extent that degradation has occurred. Experiments in which rats having thoracic duct fistulas (14) were fed labeled sitosterol and cholesterol showed sitosterol : cholesterol ratios in the lymph, from which it was deduced that sitosterol absorption was 10–15% that of cholesterol and therefore 3–6% of the sitosterol fed.

Available information thus indicates that β -sitosterol is absorbed from the intestinal tract in the human to the extent of a few per cent. The present data lend support to the results of Grundy et al. (11) that plant sterols are degraded in the intestinal tract of some human beings.

Cholesterol Absorption

Excretion of labeled cholesterol was measured in this investigation by feeding different doses of cholesterol either alone or together with labeled sitosterol. The figures obtained were corrected in the first case for losses during the extraction procedure and in the latter case

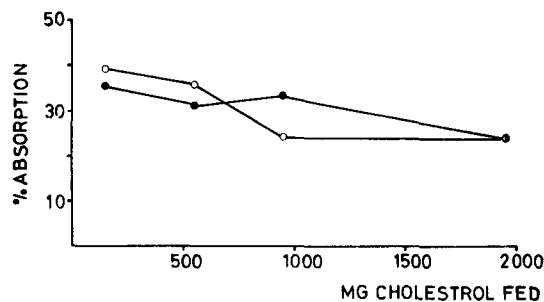


FIG. 1. Apparent absorption of different doses of labeled cholesterol fed to humans in a single test meal. The figures have been calculated from fecal recoveries of activity over the 5 days succeeding the feeding. ●, only cholesterol was fed and the recoveries were corrected by the use of an external standard in the form of β -sitosterol-22,23- ^3H added to the feces. ○, cholesterol-4- ^{14}C was fed with 150 mg of β -sitosterol-22,23- ^3H and the absorption figures were calculated by correction to a theoretical 100% sitosterol excretion.

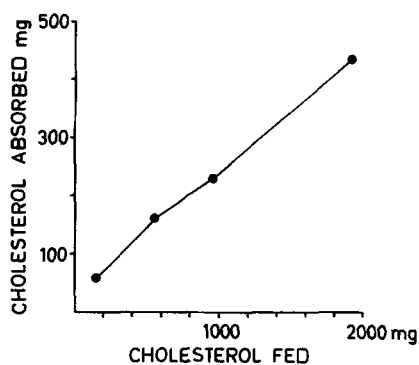


FIG. 2. Apparent total absorption of single doses of cholesterol- $4\text{-}^{14}\text{C}$ fed together with 150 mg of β -sitosterol- $22,23\text{-}^3\text{H}$. The absorption figures were calculated from fecal recoveries over the 5 days succeeding the feeding; they have been corrected to a theoretical 100% sitosterol excretion.

for losses taking place also during the intestinal transit. The results are given in Fig. 1 in terms of percentage absorption and it is obvious that the presence or absence of dietary plant sterols made little if any difference to the value obtained for cholesterol absorption (the difference was significant only for the 950 mg meal). The mean correction factor needed when sitosterol was added as external standard was 96%; when it was an internal standard, the factor was 89.9%. As some β -sitosterol is absorbed in man (4, 11), the figures for cholesterol absorption obtained by correction to a theoretical 100% β -sitosterol excretion will be somewhat too low. The use of dietary sitosterol as an internal standard for correction of cholesterol losses as suggested by Grundy et al. (11) has not proved to be of any statistical importance in the present limited number of experiments, although its importance for the individual experiment seems obvious.

Fig. 2 shows the apparent absorption of cholesterol calculated from the excretion figures corrected to a theoretical 100% fecal excretion of β -sitosterol. The absorption is almost linearly related to the dose fed. The maximal mean absorption of cholesterol is 443 mg for the subjects fed 1.91 g of cholesterol. In the three subjects fed 3×1.91 g of cholesterol (without sitosterol) in 1 day, the mean absorption was 26.6% or 1.52 g (uncorrected for cholesterol losses during intestinal transit).

In the present study the figures obtained for cholesterol absorption, calculated from fecal excretion, show the same general trend as those figures obtained earlier in the rat (9), the most characteristic feature being that the percentage absorption is largely independent of the doses fed. In other words, the results indicate that the amount of cholesterol absorbed is linearly related to the dose fed, the limiting experimental factor being the solubility of

the sterol that can be fed in a given amount of fat in a homogeneous form. The absorption curve thus has the same characteristics as those obtained in the rat (9, 14), and suggests a passive diffusion process in which saturation of the mechanism has not been reached. The results obtained do not indicate that β -sitosterol feeding should affect cholesterol absorption any differently from feeding the same amount of cholesterol. The extent of cholesterol absorption found in these studies agrees with the 36% absorption reported by Grundy and Ahrens (16) for a daily dose of 300 mg fed to a human subject.

In the rat, absorption of cholesterol, indirectly calculated from fecal excretion data (9), agrees well with that directly determined from thoracic duct transport studies (15). The lymphatic transport of cholesterol from the rat intestine can be increased fivefold over the fasting value by dietary cholesterol, the rate-limiting factor being the transfer of cholesterol from the cell to the chyle (15).

In the human there is an apparent discrepancy between cholesterol absorption indirectly calculated from fecal excretion data in single-dose isotope feeding experiments, in which the absorption seems to be directly related to the dose fed, and that calculated from steady-state isotope experiments, which indicate a maximal daily absorption of no more than 200–300 mg of dietary cholesterol (1, 2). No explanation of the different results obtained in these two types of experiments can be offered at present. The results obtained in this study are valid only for the particular type of acute loading experiments described.

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