Quantitative aspects of the intestinal absorption and metabolism of cholesterol and β-sitosterol in the rat

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ABSTRACT The quantitative aspects of intestinal absorption and metabolism of cholesterol and β-sitosterol have been studied in the rat after a single feeding of radioactive sterols.

When increasing amounts of cholesterol were fed in a constant amount of triolein, the percentage absorbed decreased only gradually and the total amounts absorbed increased to a maximum. Solubility in the fat component fed is one limiting factor in the absorption of cholesterol. At the lowest dose fed, only about 50% of dietary cholesterol was absorbed even though increasing the amount fed led to a 10- to 15-fold increase in total absorption. Sitosterol, when fed in triolein, was absorbed in amounts only one-tenth of the corresponding dose of cholesterol.

Intestinal transit studies indicate that the distinction between sitosterol and cholesterol, when fed together, took place during the process of uptake into the intestinal mucosa. Once taken up by the intestinal mucosal cells, cholesterol and sitosterol did not differ in their subsequent rate of transit out of the mucosal cell.

Feeding sitosterol with cholesterol seems to have the same effect on cholesterol absorption as feeding the same additional dose of cholesterol, the difference being that sitosterol is taken up by the intestinal wall in amounts only one-tenth to one-fifth of that of cholesterol.

The rapid and complete absorption of the triglyceride fat and the subsequent transit of the intestinal content to the large intestine are most probably important factors in the determination of the extent of absorption of nonglyceride fat. The mechanism behind the difference in extent of absorption of the closely related sterols is not explained.

KEY WORDS cholesterol . β-sitosterol . intestinal absorption . triolein . intestinal transit . oleic acid . intestinal mucosa . quantitative absorption

It is generally accepted that the absorption of cholesterol from the intestinal tract of animals and man is limited and incomplete (1–4). The quantitative aspects of this phenomenon, however, have so far not been worked out. Such information would be of interest for an understanding of the mechanism of cholesterol absorption.

Other sterols such as β-sitosterol, which is chemically very closely related to cholesterol, have been found to be absorbed to a much lesser extent than cholesterol (5, 6). Several hypotheses have been produced to explain this difference, but so far none has been convincing (1).

The present study deals with some quantitative aspects of sterol absorption in the rat in an attempt to explain specificity in the intestinal absorption of sterols.

METHODS

Male adult rats of the Sprague-Dawley strain (Anticimex, Stockholm, Sweden) were used in this investigation. Until used, they were fed on a commercial pellet diet (Anticimex).

Chemicals

Triolein was obtained from Fluka AG (Buchs, Switzerland). Sodium taurodeoxycholate was prepared according to Hofmann (7). Triolein labeled with oleic acid-9,10-^{3}H was synthesized according to standard methods. Cholesterol-4-^{14}C was obtained from the Radiochemcal Centre, Amersham, Bucks., England. Radiopurity was checked by thin-layer chromatography and was in general found to be better than 98%. Sitosterol-^{3}H was prepared from stigmasterol by hydrogenation as described (8).

Test Meal

The labeled test meal was an emulsion of triolein in a solution of skim milk powder in water. In general the labeled sterol was dissolved in the triolein oil and

emulsified in a solution obtained by dissolving 6.8 g of skim milk powder (Mazetti, Malmö, Sweden) per 100 ml of water. Emulsification was done by sonication with a Branson sonicator (Branson Instruments, Inc., Stamford, Conn.). The usual concentration of triolein in the emulsion was 100 μmoles/ml. The sterol concentration (cholesterol or sitosterol or the two in combination) ranged from 1 to 50 μmoles/ml of emulsion.

Animal Experiments

Fecal excretion of lipid-soluble radioactivity was determined after a single radiolabeled meal had been fed. To prevent coprophagy we placed the rats in restraining cages of the Bollman type (9) the night before use and for the duration of the experiment. The animals, after fasting overnight, were fed 2 ml of test meal by intubation under light ether anesthesia. Food was withdrawn for 8 hr. After that a normal pellet diet was available to the animals. Feces were collected for 4 days and the daily specimens analyzed separately in most instances.

In one series of experiments the rats were fed, for 7 days prior to the feeding of the radioisotopic test meal, a synthetic diet that was either cholesterol-free or contained 1.5% cholesterol. The base diet contained: casein, 220 g; wheat starch, 630 g; fat (triolein), 100 g; salt, 40 g; and vitamins.

In another type of experiment the animals were killed at different time intervals after the feeding of the test meal, usually at zero time, 2, 4, 6, 8, and 24 hr. The intestinal tract was divided into stomach and small and large intestine (including any feces retained). The small intestine was washed from the distal end with 25 ml of a 2.4 mM solution of sodium taurodeoxycholate to obtain intestinal content. The liver was also analyzed.

These experiments were undertaken to give information on the distribution of the sterols over the intestinal tract at different times after the feeding. Cholesterol-^{14}C and sitosterol-^{3}H were fed together in order to obtain their ratio from the same experiment.

In similar experiments we fed combinations of labeled cholesterol and sitosterol, either increasing sitosterol at a constant low cholesterol level or vice versa. In these experiments the animals were killed 4 hr after feeding and the activity data were determined for intestinal wall and liver lipids.

Each point in the curves given in the figures represents the mean value from at least four animals.

Analysis

The feces collected for a 24 hr period were homogenized in chloroform–methane 1:1 in an M.S.E. homogenizer. After a short boiling, the solution was filtered and taken to dryness. For saponification the residues were dissolved in 50 ml of methanol followed by 50 ml of 2 N NaOH. After incubation for 1 hr at 60°C the neutral lipids were extracted with light petroleum and taken to dryness.

The stomach, small intestinal wall, and large intestine plus feces were digested for 3 hr at 100°C after addition of 10 ml of solution (made from 50 g of KOH, 100 ml of water, and 300 ml of methanol) and 15 ml of methanol–water 3:1. After the addition of 12 ml of ethanol, 25 ml of water, 30 ml of ethyl ether, and 30 ml of light petroleum, the upper phase was transferred to a second funnel and washed against 50 ml of 50% aqueous ethanol; the first lower phase was again extracted with 1:1 ether–light petroleum, etc. The neutral lipids were obtained after evaporation of the collected upper phases.

Intestinal washings were extracted after the addition of equal volumes of ethyl ether, light petroleum, and ethanol as described (10).

In some experiments cholesterol was determined in the lipid extract from the small intestinal walls by the Liebermann–Burchard reaction.

Radioactivity Determinations

In general the lipid fractions were dissolved in 5 ml of chloroform, and 1 ml was transferred to a counting vial. After evaporation of the chloroform, 15 ml of scintillation fluid in toluene was added and the samples counted in a Packard model 4000 spectrometer.

The fecal extracts were usually colored and gave a high degree of quenching. For decolorization the samples were dissolved in 25 ml of chloroform and ozone was bubbled through the solution for 5–15 min. 5-ml samples were taken to dryness in counting vessels and treated as above. In this way counting could be done with minimal quenching. No losses of radioactivity could be found during ozonization.[1]

RESULTS

Sterol Absorption after a Single Radiolabeled Meal

Absorption of Cholesterol. The excretion of cholesterol-4-^{14}C in consecutive daily fecal collections from four animals is given in Table 1. It is obvious that the largest fraction is excreted already during the first 24 hr after feeding and that only small amounts are present in the feces collected during the 3rd and 4th day. A 4 day collection period is therefore satisfactory in the normal rat and furthermore, any recirculation of isotope to the intestine can only be of minor importance for the evaluation of absorption from fecal recoveries after a single feeding of radioisotope.

When cholesterol was fed in increasing amounts in the same dose of triolein (200 μmoles), the percentage

[1] Krabisch, L., and B. Borgström. Data to be published.

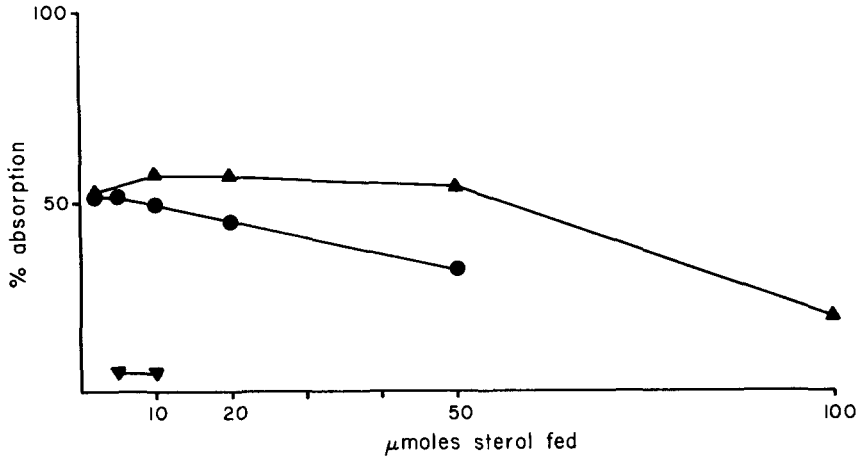

FIG. 1. Percentage absorption of cholesterol fed to rats in 200 μmoles of triolein (●) or 600 μmoles of oleic acid (▲), and of sitosterol (▼) fed in 200 μmoles of triolein. The figures were deduced from fecal excretion of isotope for 4 days after a single feeding of cholesterol-4-^{14}C. Each point corresponds to the mean of at least four separate experiments.

absorption figures shown in Fig. 1 were obtained from the fecal excretion data. It is seen that the percentage of cholesterol absorbed is almost independent of the level of cholesterol fed when this is increased 5-fold (from 2 to 10 μmoles per 200 μmoles triolein). With larger doses of cholesterol the percentage absorption gradually decreased. Even the lowest amounts of cholesterol fed were incompletely absorbed (around 50%).

When triolein was replaced by the corresponding molar amount of oleic acid (600 μmoles in 2 ml of test meal) the percentage absorbed was almost constant from 2 to 50 μmoles of cholesterol fed and then declined when the amount cholesterol was further increased.

The total amounts of cholesterol absorbed calculated from these percentages (Fig. 2) show an almost linear increase with dose, then level off. When triolein was used as carrier, the amount absorbed was lower than when oleic acid was the carrier.

When the same small dose of cholesterol (2 μmoles) was fed in different amounts of triolein—from 100 to 800 μmoles—the percentage absorption of cholesterol stayed constant.

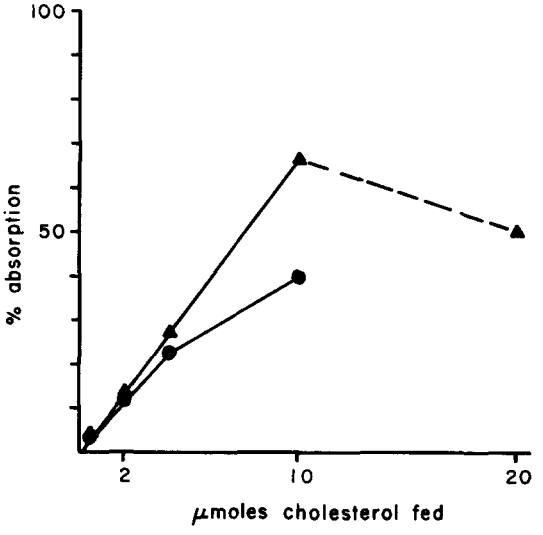

FIG. 2. Total absorption of cholesterol calculated from the percentages in Fig. 1. ●, cholesterol fed in 200 μmoles of triolein; ▲, cholesterol fed in 600 μmoles of oleic acid.

TABLE 1 EXCRETION OF CHOLESTEROL IN CONSECUTIVE DAILY FECAL COLLECTIONS FROM RATS FED CHOLESTEROL IN TRIOLEIN

Day	Rat 1	Rat 2	Rat 3	Rat 4
		% of fed dose		
1	38.4	39.3	45.9	45.6
2	8.5	3.6	3.5	5.6
3	2.8	1.5	1.1	1.5
4	3.2	0.9	0.5	0.7
Sum	52.9	45.3	51.0	53.4

Rats were fed a single meal containing 5 μmoles of cholesterol-4-^{14}C in 200 μmoles of triolein, emulsified in a solution of skim milk powder.

Absorption of Sitosterol. When sitosterol was fed in amounts of 5 and 10 μmoles per 200 μmoles triolein the percentage absorption was independent of dose and around 5% (range 0–13%).

In the experiment given in Table 2, 10 μmoles of cholesterol-4-^{14}C and of sitosterol-^{3}H were fed together in 200 μmoles of triolein to four rats and the feces analyzed. The mean absorption figures found were 42.1 and 4.7% respectively. The largest amounts were found in the first 24 hr collection. These contained approximately twice as much sitosterol as cholesterol (mean ratio 1.85); the feces collected during the 3rd and 4th day had a

TABLE 2 DAILY FECAL EXCRETION OF CHOLESTEROL-^{14}C AND SITOSTEROL-^{3}H BY RAT AFTER A SINGLE TEST MEAL CONTAINING 10 μMOLES EACH OF THE STEROLS IN 200 μMOLES OF TRIOLEIN

		% Radioactivity Recovered		
	Day	^{14}C	^{3}H	^{3}H/^{14}C
Rat 1				
	1	45.5	74.2	1.63
	2	16.6	17.8	1.07
	3 + 4	5.2	4.5	0.86
	Sum	67.3	96.5	
Rat 2				
	1	46.7	82.0	1.76
	2	8.1	8.8	1.08
	3 + 4	3.3	2.5	0.74
	Sum	58.1	93.3	
Rat 3				
	1	13.5	27.8	2.05
	2	27.7	47.2	1.70
	3 + 4	16.5	19.5	1.18
	Sum	57.7	94.5	
Rat 4				
	1	37.2	30.9	2.17
	2	8.8	12.3	1.39
	3 + 4	6.6	4.0	0.60
	Sum	52.6	97.2	

sitosterol to cholesterol ratio of 0.84 as a mean. The percentage of cholesterol absorbed was only slightly affected by the simultaneous feeding of an equal dose of sitosterol when it is related to the total amount of sterol fed (cholesterol + sitosterol). Absorption of cholesterol when fed in a dose of 20 μmoles per 200 μmoles triolein was 45.2% compared to 42.1% when 10 μmoles of cholesterol was fed together with 10 μmoles of sitosterol.

Transit of Cholesterol and Sitosterol in the Gastrointestinal Tract of the Rat

Fig. 3 shows the distribution of cholesterol-4-^{14}C and sitosterol-^{3}H in the stomach, small intestinal content, small intestinal wall, large intestine (including feces where present), and liver at different times after the feeding of a test meal containing 10 μmoles each of sitosterol and cholesterol in 200 μmoles of triolein. The stomach is emptied by 4 hr after feeding; during this time an approximately constant amount can be recovered from the intestinal content. There is a higher level of sitosterol in the intestinal content. The reverse is the case in the intestinal wall, which contains around 30% of the cholesterol and only 5–10% of sitosterol during the first 4 hr. The large intestine starts to receive radioactivity during the 2nd hr of feeding and shows a rapid increase during the next few hours; thereafter a slower increase takes place up to the 24 hr interval. At all times there is more sitosterol than cholesterol in the large intestine. At the 24 hr interval the large intestine contains around 40% of the fed cholesterol radioactivity and almost double the percentage of sitosterol radioactivity. The

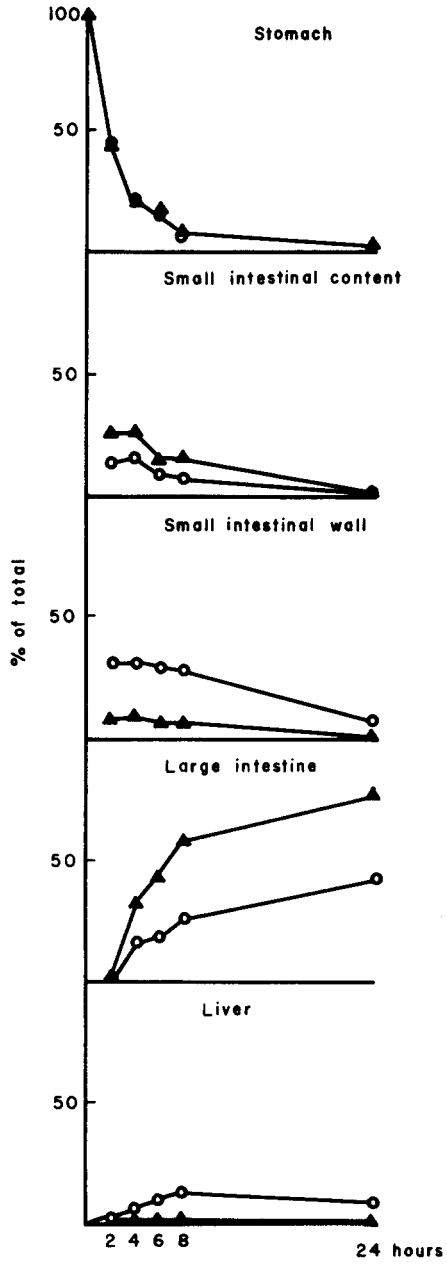

FIG. 3. The distribution of cholesterol and sitosterol in different parts of the gastrointestinal tract and liver of the rat at different times after the feeding of a test meal containing 10 μmoles each of cholesterol-4-^{14}C (○) and sitosterol-^{3}H (▲) in 200 μmoles of triolein. Each point is the mean from two experiments except the 24 hr figures, which are the mean of eight experiments.

relation between cholesterol and sitosterol transit is most clearly expressed in the ratios of sitosterol/cholesterol radioactivity given in Fig. 4. In the stomach the ratio is close to 1.0 (i.e., that of the test meal) as long as significant activities can be found. In small intestinal content the ratio varied between 1.43 and 1.94 with no characteristic change with time. In the intestinal wall the ratio varied between 0.21 and 0.36, which indicates a

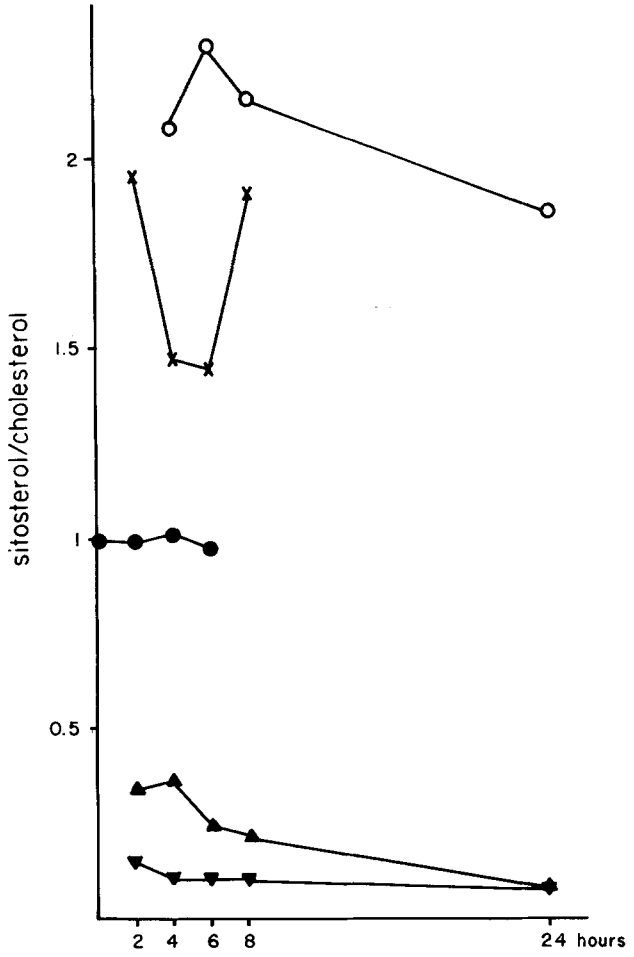

FIG. 4. The sitosterol/cholesterol ratio in different parts of the gastrointestinal tract and liver of the rat at different times after a test meal containing 10 μmoles each of cholesterol-4-^{14}C and sitosterol-^{3}H. The figures are derived from those given in Fig. 3. ●, stomach; ×, small intestinal content; ▲, wall of the small intestine; ○, large intestine; ▼, liver.

higher uptake of cholesterol than sitosterol. In the large intestine the ratio is around 2. A slight decrease takes place from the 8th to the 24th hr. The number of observations are too small to give any significance to this difference. In the liver the ratio is 0.10–0.12.

In similar experiments with triolein-^{14}C it was seen that the absorption of this labeled triglyceride in the intestinal tract is rapid. At no time is there any appreciable accumulation in intestinal content or intestinal wall. Absorption is 95% or more.

Uptake of Dietary Cholesterol and Sitosterol by the Small Intestinal Mucosa

The previous results showed that during the absorption period the small intestinal wall incorporated significant amounts of dietary cholesterol and smaller amounts of sitosterol. The uptake of these sterols into the small intestinal wall during the digestion and absorption processes was then studied in more detail. Test meals with different amounts of the sterols in combination were fed to rats that were killed 4 hr later. In Fig. 5 are shown the amounts of labeled sterol in the small intestinal wall 4 hr after feeding, as calculated from isotope data. In the experiments given in the upper part of the figure (A) increasing amounts of sitosterol were fed with a constant dose of cholesterol; in those shown in the lower part (B), increasing amounts of cholesterol were fed with a constant level of sitosterol. The figures have been calculated as: amount of sterol found in intestinal wall as a percentage of the amount of sterol that had left the stomach at this time. It is seen that the percentage of cholesterol present in the wall goes down as the dose is increased, either with cholesterol or sitosterol. The curve for

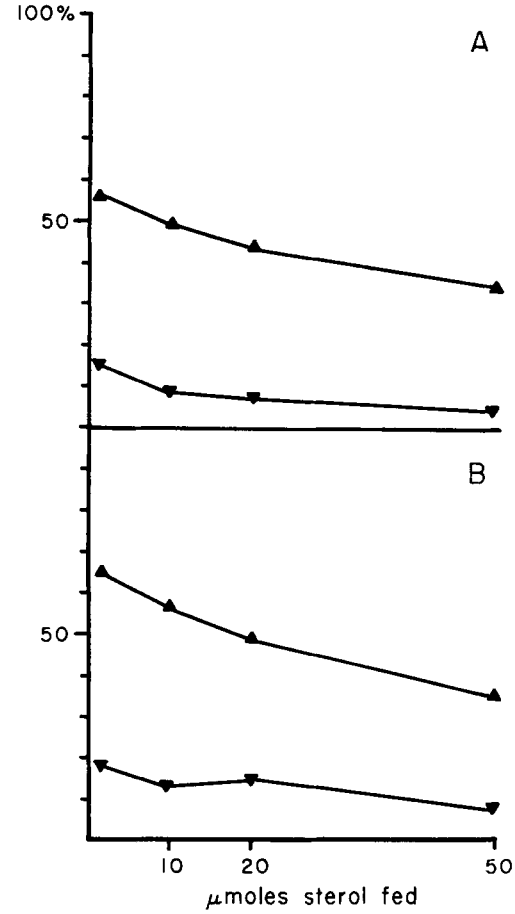

FIG. 5. Cholesterol-4-^{14}C (▲) and sitosterol-^{3}H (▼) in the small intestinal wall of rats 4 hr after they were fed a test meal containing the sterols in 200 μmoles of triolein. The figures are percentages of the amount of isotope that had left the stomach at 4 hr. Part A represents experiments in which increasing amounts of labeled sitosterol (2–50 μmoles) were fed with a constant dose of cholesterol (2 μmoles) in 200 μmoles triolein. The curves in part B refer to experiments in which increasing amounts of cholesterol (2–50 μmoles) were fed with a constant amount of sitosterol (2 μmoles) in 200 μmoles of triolein. Each point on the curves refers to the mean of two experiments.

cholesterol uptake in the wall is quite similar to the one obtained for cholesterol absorption in Fig. 1. The sitosterol curves have a similar course but at a much lower level. The ratio sitosterol/cholesterol is between 0.12 and 0.30. Substituting cholesterol for sitosterol or vice versa does not appreciably affect the shape of the curves. The total amounts of cholesterol and sitosterol present in the intestinal wall (calculated from the radioactivity) increase for both sterols with increasing total amount of sterol fed (Fig. 6).

When both the mass and radioactivity of cholesterol in the intestinal wall were correlated 4 hr after meals with increasing cholesterol content had been fed, the results in Fig. 7 were obtained. It is seen that the total amount of cholesterol in the wall increases in parallel with the increase in dietary (labeled) cholesterol, and that the difference between total cholesterol and dietary cholesterol stays constant except for the lowest dose of cholesterol fed. This difference can probably be ascribed to the fact that the dietary cholesterol is diluted in the lumen with endogenous cholesterol so that the actual uptake is greater than that calculated from the isotope data. The relative importance of this dilution will decrease as the amount of dietary sterol increases.

Feeding rats cholesterol-rich food for 1 wk did not influence the amount of total cholesterol in the intestinal wall, nor the uptake of labeled cholesterol from a test meal (see Table 3). In these experiments, the test meal was fed and sterol was determined after an overnight fast.

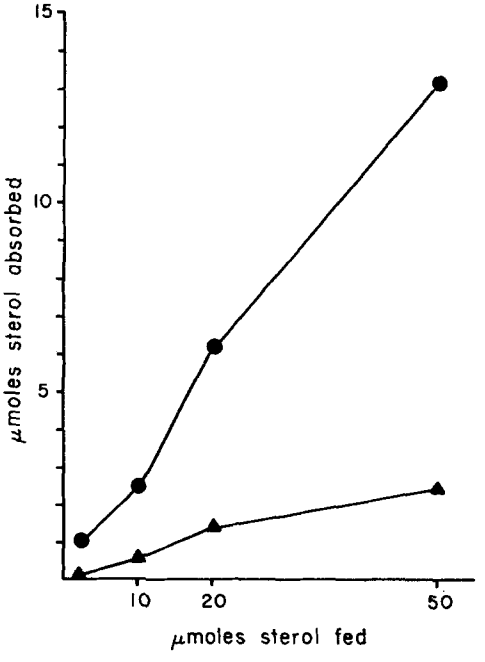

FIG. 6. Total amounts of sterol present in the intestinal wall of experiment described in Fig. 5; values calculated from the isotope data. ●, cholesterol; ▲, sitosterol.

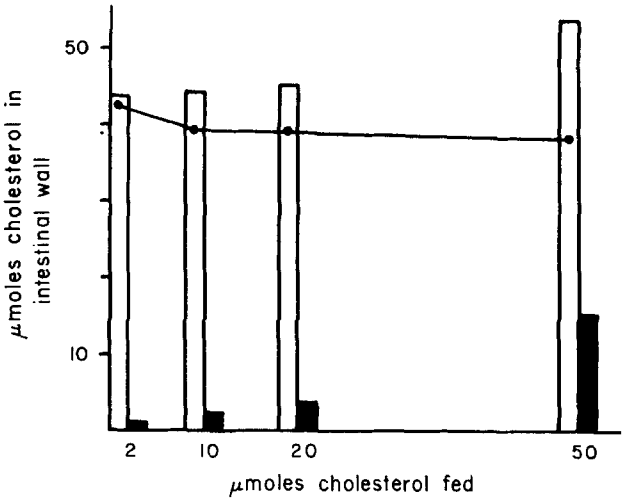

FIG. 7. Mass and radioactivity of cholesterol in the small intestinal wall of rats 4 hr after they were fed a test meal containing different levels of cholesterol-4-^{14}C in 200 μmoles of triolein. Open bars are cholesterol content obtained by chemical determination. Filled bars are amount of dietary cholesterol present in the intestinal wall, calculated from the radioactivity of the cholesterol fed and of cholesterol extracted from the intestinal wall. Each figure is the mean of five different experiments. ●—● represents mass minus activity.

TABLE 3 EFFECT OF PRIOR CHOLESTEROL FEEDING ON INTESTINAL UPTAKE OF CHOLESTEROL FED IN TRIOLEIN

	Cholesterol in Previous Diet	
	1.5%	None
Total cholesterol in wall (*μmoles*)	48.5 ± 2.6	45.2 ± 1.0
Test meal cholesterol taken up (*μmoles*)	5.2	5.4
Test meal cholesterol taken up (%)	52.2 ± 3.2	53.7 ± 2.4

Two groups of five rats each were fed a cholesterol-free diet or the same diet with 1.5% cholesterol added for 7 days. 24 hr after the last feeding they were given by gastric intubation 2 ml of a test meal containing 10 μmoles of cholesterol-^{14}C in 200 μmoles of triolein. 4 hr later they were anesthetized with ether and the small intestine was removed and washed with 25 ml of 2.4 mM sodium taurodeoxycholate. Cholesterol mass and radioactivity were determined.

The uptake of cholesterol into the intestinal wall of rats with external bile fistulas was found to be less than 3% of the dose fed (5 μmoles in 200 μmoles triolein) during the whole absorption period (from 2 to 24 hr after feeding).

DISCUSSION

The absorption data of the present investigation have been obtained from experiments involving a single feeding of isotope and have been based on fecal excretion of the isotope. The interpretation of the results obtained from such experiments is not always straightforward, especially not with compounds undergoing an entero-

hepatic circulation. It is not a priori evident that isotope movement is equivalent to mass movement. One obvious possibility is that recirculation of absorbed labeled cholesterol will affect the fecal excretion data. The figures obtained and given in Table 1 and also in Fig. 3, however, indicate that the intestinal transit in the rat is rapid and that the isotope excretion during the 3rd and 4th day after feeding is small compared to direct excretion of unabsorbed material. Furthermore, Wilson has shown (11) that the fecal excretion of intravenously injected cholesterol does not exceed 10% in a 4 day period. The reexcretion of absorbed cholesterol in our experiments will tend to lower the figures for absorption of cholesterol; the magnitude of this effect, however, will certainly be less than 10% of the amount absorbed.

The use of balance studies for measuring sterol absorption is based on the assumption that no degradation of sterols to a form that is not recovered by the method of analysis used occurs in the intestinal tract. Recent evidence in the human, however, indicates that under certain conditions considerable quantities of sterol can be degraded in the intestinal tract by intestinal bacteria (2, 12). The data in Table 2, which show almost complete recovery of sitosterol activity, indicate that this factor has been of little or no importance in the present experiments.

The absorption figures obtained when increasing amounts of cholesterol were fed in a constant dose of triolein show that even the lowest amount of cholesterol fed is absorbed only to the extent of 50%. Increase in dose results in a gradual decrease in absorption when calculated on a percentage basis. The total amount of cholesterol absorbed, however, increases sharply with increased dose. The solubility of cholesterol in triolein sets a limit to the amount that can be fed in the emulsified form. This solubility is around 10 μmoles/100 μmoles of triolein (13) and it is exceeded in the highest dose used in Fig. 3. The solubility of cholesterol in oleic acid is, however, approximately 5–6 times that in triolein (13). Feeding cholesterol in oleic acid also gives higher maximal absorption figures.

The paradox of these results is the incomplete absorption of even small doses of dietary chloesterol and the much greater maximal capacity of absorption. Thus from 2 μmoles of cholesterol fed, only 1 μmole is absorbed while the total absorption from 50 μmoles (in oleic acid) is 15 μmoles.

The mechanism behind this effect is not known at the present time. If dietary cholesterol were to mix in the intestinal lumen with an endogenous cholesterol pool that was large in relation to the amount of dietary cholesterol, an effect similar to the one observed could be expected. In this case it would also be necessary that the pool of endogenous cholesterol be large enough to saturate the absorption capacity of cholesterol. The main source of intraluminal cholesterol in the rat is the cholesterol of bile (11). The total flux of bile cholesterol in the rat, however, is less than 0.5 μmole/hr and could not alone be responsible for this effect.[2]

Intestinal content during digestion of a fat-containing meal consists of a dispersion of an oil phase in a micellar phase (10), the latter being the physical form in which lipids are taken up by the intestinal mucosa. The uptake into the cell membrane therefore would be related to the concentration in the micellar phase. In vitro studies have shown that in a system resembling that present in the intestinal content, lipids distribute between the oil phase and the micellar phase; the partition ratio is largely independent of the concentration in the system (8). The micellar concentration of cholesterol in intestinal content therefore would be expected to be directly related to the concentration of cholesterol in the dietary fat, and the uptake into the cell membrane would similarly be related to the concentration in the dietary fat. Such a mechanism could be the basis of the results obtained in vivo.

Earlier studies have shown that transit of cholesterol from the intestinal mucosa was a slow process relative to the uptake into the mucosa from the intestinal lumen (14). Cholesterol accumulated in the intestinal mucosa during the absorption period.

An attempt was therefore made in this investigation to measure the uptake of dietary sterol into the wall of the intestinal mucosa as an indication of absorption 4 hr after feeding. The technique used involves the flushing of the intestinal lumen with a bile salt solution to remove dietary sterol present outside the cells. It is recognized that such a procedure cannot give a complete separation of what is outside and inside the cell and is bound to give too high figures for cell wall activity. When cholesterol uptake in the wall was calculated as a percentage of what had left the stomach after 4 hr, the figures obtained for absorption were somewhat higher than those from fecal excretion data (compare Figs. 1 and 5).

The results obtained show that the total mass of cholesterol in the wall is increased in proportion to the labeled cholesterol taken up. Uptake of label into the wall thus represents actual uptake of exogenous cholesterol and not exchange.

The figures obtained for sitosterol absorption calculated from fecal excretion data (absorption being defined as the difference between the radioactivity fed and that recovered in feces) indicate an absorption of 0–13%, with a mean of 5% of the dose fed, whether this was 5 or 10 μmoles. Absorption of sitosterol thus was around

[2] Based on analysis of rat bile from cannulated rats in which bile salt was continuously infused into the small intestine.

10% of that of cholesterol when fed in the same amounts. If the sterol present in the liver in these experiments represents the sterol mixture transported from the intestine in the chyle, the calculated ratio sitosterol/cholesterol absorbed is 0.13, in quite good agreement with the absorption data. More definite figures for the ratio sitosterol/cholesterol transported in the chyle have to await results from experiments in rats with thoracic duct fistulas.

The absorption figures for sitosterol obtained here agree with those estimated by analysis of rats for absorbed sterols (retention in the body [5] or recoveries in thoracic duct lymph [6]) but are in contrast to the much higher figures earlier obtained in balance studies by several investigators (6, 15–18). In some of these studies degradation (to nondigitonin-precipitable substances or phenols) may be responsible for the low fecal recoveries. Another factor to explain the low recoveries may be the use of crude plant sterols—both labeled and unlabeled—including the 24-methyl homologue of sitosterol, which might have a different metabolic pattern.

The ratio sitosterol/cholesterol in the large intestine 24 hr after feeding was found to be 1.86 (mean of eight experiments). If it is assumed that cholesterol absorption in these experiments is around 50% (Fig. 1, 10 μmoles sterols/200 μmoles triolein) and the sitosterol absorption is 5%, then the ratio of unabsorbed sterols would be 95/50 = 1.90, a figure in good agreement with the figure found for fecal sterols and those of the large intestine.

Uptake of sitosterol into the intestinal wall during absorption seems to follow the same pattern as that for cholesterol, only on a considerably lower level: the ratio sitosterol/cholesterol in the wall is between 0.20 and 0.35 during the active absorption phase. That these figures are higher than those for the relative absorption of the two sterols could well be explained by the difficulty in separating what is outside and inside the wall, for the ratio in intestinal content is something like 10 times as high as in the sterol mixture transported in the chyle.

Sitosterol affects cholesterol uptake by the intestinal wall in the same way as a corresponding dose of cholesterol, but is absorbed to a much lesser extent. Total sterol transport from the intestine in the chyle will therefore be decreased when part of the sterol fed is exchanged for phytosterols. The total amount of sitosterol taken up by the intestinal wall, however, increases almost directly in proportion to the amount fed. In this respect, also, sitosterol absorption is similar to cholesterol absorption.

The constancy of the sitosterol/cholesterol ratio in the intestinal wall with time indicates that, once taken up by the intestinal cell, the sterols do not differ in their rate of transit out of the cell. The results do not support a mechanism dependent on difference in intestinal metabolism as a factor in the specificity of sterol absorption (1). In this respect they agree with the finding that the sitosterol/cholesterol ratios are identical in the different fractions of intestinal mucosa homogenates isolated by gradient centrifugation (19).

The results obtained seem to lend support to the idea that the specificity of sterol absorption is to be found at the level of uptake into the cell membrane. Glover and Morton (1) have concluded that the block in phytosterol absorption "appears to be the transport of the sterol from the lumen into the mucosa cell." As a possible mechanism for this block they suggest the slow transfer of phytosterol relative to cholesterol in a series of steps, including the reaction of phytosterol with an animal type of lipoprotein. In this way each successive change for a phytosterol molecule would become more difficult as it penetrates the cell, in which the lipoproteins are already loaded with cholesterol.

An earlier in vitro experiment has shown that there is no difference in the partition of the two sterols between an oil and micellar phase in a system resembling that to be expected in the intestinal content during fat digestion (8). The concentrations of sitosterol and cholesterol in the micellar solution of intestinal content therefore can be expected to be related to their concentration in the fat fed. In spite of this, the ratio of sitosterol to cholesterol taken up into the cell is probably only one-tenth of this.

In in vitro experiments with intestinal slices or everted sacs the two sterols are taken up in the same ratio as the ratio of their concentrations in a micellar solution used as incubation medium. Possible differences in the results of in vitro and in vivo experiments in respect to sterol absorption have recently been discussed (20).

Localization of the specificity step of sterol absorption to a specific physicochemical or chemical reaction is therefore not possible at present.

An interesting piece of information that can be obtained from the transit experiments of this investigation is the rapid delivery of the unabsorbed sterols to the content of the large intestine. It is important to recognize this transit time as a limiting factor in the time available for absorption. The experiments with labeled triglycerides show that they are rapidly and completely absorbed from the lumen of the small intestine and leave the unabsorbed sterols behind in the intestinal content. The uptake of sterols by the intestine is a rather rapid process that seems parallel to and largely dependent on absorption of triglyceride fat.

As would be expected from earlier results showing the dependence on bile for cholesterol absorption (21), no significant uptake of cholesterol into the intestinal wall was found in rats with bile fistulas.

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