Sterols in the small intestine of the rat, guinea pig, and rabbit

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ABSTRACT The sterol content and composition of the small intestinal mucosa has been studied in the rat, guinea pig, and rabbit. In the three species, noncholesterol sterols formed a large proportion of the total sterol content. In the rat, the contribution due to plant sterols was much smaller than in the guinea pig and rabbit, but in all three species the amount of campesterol present seemed disproportionately large compared with β-sitosterol. These observations are presented as evidence that the intestinal mucosa is able to discriminate in its uptake of sterols from the lumen. The presence of plant sterol at all levels of the intestine makes it clear that luminal sterols can be taken up throughout the small bowel, but the data suggest that the upper intestine is the major site of sterol uptake.

In the rat, the sterol composition of the intraluminal contents was also studied after its separation by ultracentrifugation into an aqueous (or micellar) phase and a sediment. Examination of the content and composition of the intraluminal sterols at different levels of the intestine provided support for the idea that the major site of cholesterol absorption was in the upper small intestine. The major part of the intraluminal sterol was found in the sediment; precipitation did not, however, render sterols unavailable for absorption and the micellar sterols reflected closely the pattern found in the corresponding sediment.

Lathosterol was present in the lumen at all levels of the small intestine. Evidence is presented that the small intestine was not a major source of this material, which was probably derived from skin. Despite the presence of lathosterol in the intestinal lumen, it would appear that, at least in the rat, mucosal lathosterol is derived largely from local synthesis.

SUPPLEMENTARY KEY WORDS cholesterol · lathosterol · plant sterols · mucosal sterols · luminal sterols · sterol absorption · tritiated digitonin assay · gas–liquid chromatography · mass spectrometry

THE METABOLISM of cholesterol in man and other mammals has been the subject of numerous experimental studies. In many of these it has been assumed that other sterols are of no importance in the interpretation of the results. Often this assumption is reasonable, for in many mammalian tissues sterols other than cholesterol are present only in minute amounts. In skin, hair, and feces, however, large amounts of noncholesterol sterols are found, and serious errors might result if the sterol content were assumed to be cholesterol alone (1).

It has been recognized for many years that noncholesterol sterols are readily detectable in the small intestinal mucosa. Plant sterols such as campesterol and β-sitosterol are present in large amounts in the diets of man and most laboratory animals; several workers have shown that such compounds can be taken up into the mucosa of the small intestine even though their subsequent transport into the lymph is markedly impaired when compared with that of cholesterol (2–6). It has also been demonstrated that precursors of cholesterol such as lathosterol and 7-dehydrocholes-

Abbreviations: DPS, digitonin-precipitable sterols; GLC, gas–liquid chromatography; GLC–MS, combined gas–liquid chromatography and mass spectrometry. The following systematic names are given to sterols referred to in this paper by trivial names: cholestanol, 5α-cholestan-3β-ol; cholesterol, cholest-5-en-3β-ol; lathosterol, 5α-cholest-7-en-3β-ol; 7-dehydrocholesterol, cholesta-5,7-dien-3β-ol; coprostanol, 5β-cholestan-3β-ol; desmosterol, cholesta-5,24-dien-3β-ol; methostenol, 4α-methyl-5α-cholest-7-en-3β-ol; lanosterol, 4,4,14α-trimethyl-5α-cholesta-8,24-dien-3β-ol; dihydrolanosterol, 4,4,14α-trimethyl-5α-cholest-8-en-3β-ol; campesterol, 24α-methyl-cholest-5-en-3β-ol; stigmasterol, 24β-ethyl-cholesta-5,22-dien-3β-ol; β-sitosterol, 24β-ethyl-cholest-5-en-3β-ol.

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terol occur in the mucosa of the guinea pig, rat, and other animals (7–11).

Despite these observations there have been few investigations on the noncholesterol sterols of the small intestinal mucosa. The main reason for this has been the inadequacy of most methods for the characterization of sterol mixtures. In the present study, tritiated digitonin assay, GLC, and GLC–MS have been used for quantitative and qualitative assessment of the sterols of the small intestine of the rat, guinea pig, and rabbit. The sterol composition of the luminal contents of the rat intestine was also investigated.

MATERIALS

Animals

Rats were male and female animals of the Charles River CD strain, weighing 190–330 g; in studies of luminal contents larger animals (450–550 g) were used. English short-haired female guinea pigs (380–510 g) and New Zealand white rabbits (1890–2270 g) were also studied. Rats were fed Purina laboratory chow; guinea pigs and rabbits were given Purina laboratory rabbit chow. Three rats were fed rabbit chow for 2 wk before they were killed. In most studies animals were allowed food ad lib.; fasting rats were deprived of food only for the night before they were killed. In order to minimize coprophagy the cages were floored with a wire mesh to permit the passage of feces. Rats and guinea pigs were killed by a blow to the head; rabbits were given a lethal dose of pentobarbital.

Authentic Sterols and Radioactive Materials

Cholesterol, cholestanol, and 7-dehydrocholesterol were obtained from Calbiochem, Los Angeles, Calif.; the 7-dehydrocholesterol was purified by recrystallization from methanol on each occasion before use. Coprostanol and dihydrolanosterol (batch 5168, pure by GLC) were obtained from Steraloids, Pawling, N.Y. Campesterol, stigmasterol, and β-sitosterol were donated by Dr. Andrew Rozanski of Upjohn Co., Kalamazoo, Mich. Lathosterol, methostenol, and desmosterol were gifts from Dr. George Schroepfer and Dr. W. W. Wells.

Lanosterol, which contained a large amount of dihydrolanosterol as an impurity, was obtained from Mann Research Laboratories, New York. It was purified by conversion to the acetate dibromide (12). After two crystallizations from methanol–acetone, the regenerated lanosterol (mp 137–139°C) still contained 5% dihydrolanosterol as judged by GLC.

Tritiated digitonin for sterol assay was prepared by New England Nuclear Corp., Boston, Mass. Digitonin (Sigma Chemical Co., St. Louis, Mo.) was exposed to tritium gas using the Wilzbach technique. The labeled material was purified by repeated precipitation with cholesterol. The cholesterol digitonide was cleaved by solution in pyridine; an excess of ether was added and the precipitate of digitonin was collected by centrifugation. Cholesterol-4-^{14}C and cholesterol-4-^{14}C oleate were also supplied by New England Nuclear and repurified by column chromatography before use.

Dietary Fat and Sterol Composition

The fat content of Purina laboratory chow was quoted as 4.3% and that of laboratory rabbit chow as 2.4%. The sterol content and composition of both kinds of chow were measured by tritiated digitonin assay, GLC, and GLC–MS.

Rat chow contained approximately 3.37 μmoles of DPS per gram, of which 2.12 μmoles were free sterol and 1.25 μmoles were esterified. Rabbit chow contained 2.78 μmoles/g, of which 1.76 μmoles were free sterol and 1.02 μmoles were esterified.

The cholesterol in the rat chow accounted for approximately 25% of the total sterols (Fig. 1). The other major peaks in the sterol chromatogram were due to campesterol, stigmasterol, and β-sitosterol; following the β-sitosterol peak there were at least three smaller peaks which could not be characterized by comparison with available authentic sterols. The cholesterol peak of the free sterol fraction was approximately 31% of the total sterol area, and in the ester fraction it was about 21%. In the rabbit chow, cholesterol was much less prominent and accounted for only 3–4% of the total sterol area (Fig. 2), with no obvious difference between the free and ester fractions.

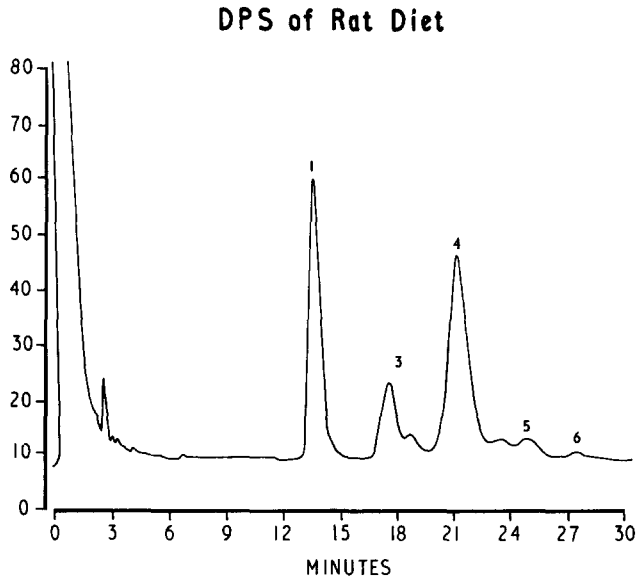
METHODS

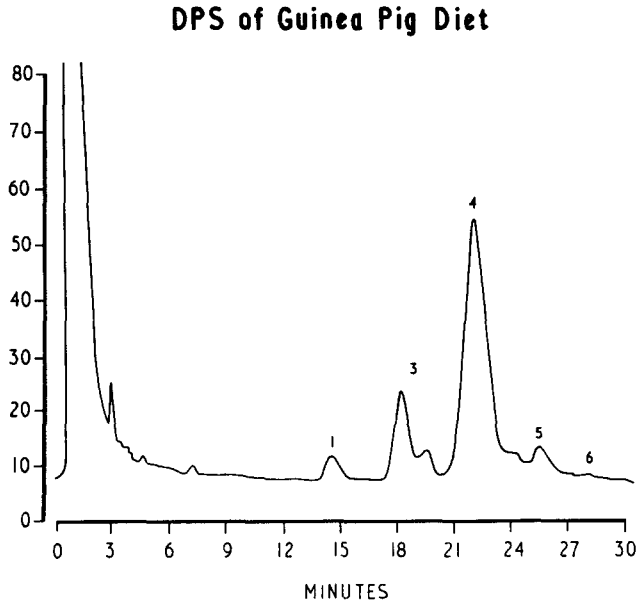
Preparation of Mucosa and Extraction of Lipids

The small intestine from the entry of the bile duct to the end of the terminal ileum was removed, washed through with saline, and divided into three portions of approximately equal length. After gentle blotting, the mucosa was scraped off the muscle layer with the edge of a glass slide. In the rat and guinea pig the entire mucosa of each third of the intestine was used. In the rabbit a large sample was taken from the middle of each third.

The mucosa was weighed and homogenized in normal saline. An aliquot was extracted by the method of Folch, Lees, and Sloane Stanley (13). Before separation into two phases, trace amounts of cholesterol-4-^{14}C and cholesterol-4-^{14}C oleate were added as internal standards for estimation of the final recovery of free and esterified sterols.

The lipids of the lower phase were applied to a column containing 2 or 3 g of silicic acid (Bio-Sil BH, 100–120





FIGS. 1 AND 2. Gas–liquid chromatograms illustrating the composition of digitonin-precipitable material in rat (top) and rabbit (bottom) chow after hydrolysis to convert all sterols into an unesterified form. Details of chromatographic technique are presented in the text. Peak *1* is cholesterol. Peak *3* includes campesterol (the larger peak) and stigmasterol, and peak *4* is β-sitosterol. The peaks following β-sitosterol were not identified.

mesh; Bio-Rad Laboratories, Richmond, Calif.). Sterol esters were eluted with 40 ml of 2% ether in hexane, and free sterols were eluted with ether; the ester fraction was then saponified in ethanolic KOH, and the resulting free sterols were extracted into hexane. The ether extracts from the column and the hexane extracts following saponification were dried under nitrogen and redissolved in ethanol–acetone 1:1. An aliquot was taken for liquid scintillation counting in order that an estimate of recovery of the labeled materials added initially could be made. A much larger aliquot was then used for quantitation of DPS.

Luminal Sterols

The rats were allowed to feed ad lib. until they were killed. The peritoneum was opened and clips were placed to occlude the lumen at various positions of the small intestine to prevent shift of luminal contents. The small intestine was divided into three approximately equal parts and the luminal contents were collected. Material from 2 or 3 animals was pooled in cellulose nitrate ultracentrifuge tubes and kept in a water bath at 70°C from the time of addition of the material from the first animal until 15 min after addition from the last; this was done to inactivate pancreatic lipase and cholesteryl ester hydrolase. The tubes were then placed in an incubator at 37°C until the material from all groups of animals to be studied on that day had been collected. The tubes were then ultracentrifuged for 4 hr at 110,000 *g*. Refrigeration was not used and the temperature in different experiments varied between 20 and 30°C. The material separated into a dark green-brown sediment and a clear supernatant of a similar, but lighter, color. A measured aliquot of the clear supernatant was withdrawn; the residual supernatant was removed and discarded after its volume had been measured. The aliquot of supernatant fluid was extracted with chloroform–methanol 2:1. The material was not saponified, and the amount and composition of free sterol were measured following digitonin precipitation. No oil phase was seen on the surface of the supernatant after ultracentrifugation of the intestinal contents, and no evidence was found for cholesterol enrichment of the surface layer.

The sediment was saponified with ethanolic KOH, and the sterols were extracted with hexane. In a separate study on four animals, designed to measure the free:ester ratio of the luminal sterols, the sediment from the lower half of the small intestine was taken and the lipids were extracted with chloroform–methanol 2:1. The sterol esters were separated from the free sterols by column chromatography, and the amount of each was measured by digitonin precipitation. The proportion of different sterols present in the free and ester fractions was assessed by GLC.

Shed Epithelial Debris

In order to obtain shed epithelial debris, isolated jejunal or ileal loops were constructed in six rats. The lumen was open to the exterior at both ends of the loop. The animals were killed 18–24 hr later; the debris in the lumen of the loops was removed and the sterols were extracted and prepared for GLC.

ANALYTICAL METHODS

Sterol Assay

Total DPS were measured using a modification of the method of Morris (14). 1 vol of a 0.5% solution of tritiated digitonin in 50% ethanol was added to 2 vol of ethanol–acetone 1:1 in which the sterols were dissolved. After standing overnight the precipitated sterol digitonide was collected by centrifugation, washed with 0.4 vol of methanol–ethanol–water 4:1:5, ether–acetone 2:1, and finally with pure ether. When the precipitate was dry, it was dissolved in 2 ml of absolute methanol. A 0.5-ml aliquot was added to 15 ml of toluene containing 4 g of 2,5-diphenyloxazole and 50 mg of 2,2-*p*-phenylenebis(5-phenyloxazole) per liter. Radioactivity was measured in a Beckman LS 250 liquid scintillation counter. The disintegrations due to tritium were corrected for the cholesterol-^{14}C present as an internal standard. The quantity of sterol present was estimated using a standard curve constructed from duplicate determinations of the amounts of tritiated digitonin precipitated with known amounts of cholesterol. The amount of sterol originally present was obtained after correction of this figure for recovery of the internal standard. The remaining 1.5 ml of the methanol solution of sterol digitonide was evaporated to dryness and used for GLC.

The tritiated digitonin assay was tested for its applicability to sterols other than cholesterol. 7-Dehydrocholesterol, lathosterol, cholestanol, methostenol, desmosterol, campesterol, stigmasterol, and β-sitosterol gave standard curves very close to that of cholesterol, i.e., they precipitated almost identical amounts of tritiated digitonin on a molar basis. Coprostanol, lanosterol, and dihydrolanosterol precipitated poorly under the conditions used in the assay, and digitonin precipitation is an inadequate method for their measurement. The precision of this assay was good with large amounts of cholesterol; with 250 μg the standard deviation of multiple replicates was ± 1.3%, but with 25 μg the standard deviation was ± 4.1%. With smaller amounts, difficulties in handling the precipitate led to a decrease in accuracy.

Gas–Liquid Chromatography

Sterol digitonides were cleaved by pyridine prior to GLC. An excess of ether was added and the precipitate of digitonin was removed by centrifugation. The ether–pyridine solution was evaporated to dryness and the sterols were redissolved in ether or dimethylformamide for injection into the gas chromatograph.

GLC was performed on a Barber-Colman series 5000 apparatus with a hydrogen flame detector; 6-ft U-shaped columns (I.D. 4 mm) were used. The stationary phase was 3% OV-17 on 100–120 Chromosorb W (AW, DMCS, high performance; Supelco, Inc., Bellefonte, Pa.). The column temperature was held at 280°C; both the flash heater and the detector were at 320°C. The nitrogen inlet pressure was 24 psi and the outlet was at atmospheric pressure.

Preliminary identification of the compounds was carried out by comparison of their retention times with those of authentic sterols. Further identification of the major components of representative samples was done using GLC–MS.

Peak areas were measured three times with a planimeter. The relationship of the detector response to the amount of sterol injected was tested for cholesterol and campesterol and shown to be linear. The detector response was not identical for different sterols either on a unit weight basis or on a molar basis, but the difference was not greater than 10% for any of the sterols tested. Although this discrepancy would introduce a relatively small error into the measurement of individual sterols in a mixture, we have preferred to express our results for individual peaks as the percentage area which they occupy of the total sterol chromatogram.

As the resolution between certain sterols was poor, we have not used the term "peak" to imply the presence of a single compound but rather to describe "areas" of the chromatogram which could clearly be distinguished from other peaks. Peak 1 was largely cholesterol, but cholestanol would also appear in the same area. The area covered by peak 2 overlapped the retention times of lathosterol, 7-dehydrocholesterol, and desmosterol, while peak 3 included campesterol, methostenol, stigmasterol, and dihydrolanosterol. Peak 4 included β-sitosterol and lanosterol. Peaks 5 and 6 could not be characterized by comparison of their retention times with available authentic sterols. One surprising feature was the virtual absence of coprostanol in the mucosal sterols of all three species and in the luminal sterols of the rat.

The reproducibility of planimetry was excellent for large peaks, but there was considerable error for small peak areas. We agree with D'Hollander and Chevallier (15) that the measurement of peaks with a percentage area less than 2% of the total might be attended by an error approaching 100%. Despite these necessary reservations, planimetry appeared satisfactory for the comparisons made in this study; almost all the differences noted in the Results section were obvious on simple inspection of the chromatogram.

Combined Gas–Liquid Chromatography and Mass Spectrometry

GLC–MS was carried out on an LKB 9000 instrument. The stationary phase was 1% OV-17 on Gas-Chrom

Q (Applied Science Laboratories Inc., State College, Pa.). The column temperature was 230°C. Samples in the solid state, absorbed on stainless steel gauze, were injected into the flash heater at 250°C. The carrier gas was helium with a flow rate of 30 ml/min. The molecular separator was maintained at 250°C and the ion source at 270°C. The mass spectrometer ionizing current was 50 μa, and the ionizing energy was 70 ev during the mass spectral scans. Identification of sterols was made by comparison of their spectra with those of available authentic compounds, with confirmation from study of previously published spectra (16–18). Peaks 5 and 6 in the guinea pig mucosa could not be identified by these methods. Peak 5 was produced by a compound with a molecular weight of approximately 414, which probably had a 3β-OH, Δ^5 configuration. The material in peak 6 had a molecular weight of approximately 392, but its fragmentation pattern did not suggest that it was a 3β-OH sterol.

RESULTS

Mucosal Sterols

Content. The mean values for the free and esterified sterol content of the small intestinal mucosa of the rat, guinea pig, and rabbit are presented in Table 1. In the rat, measurements were made on both fed and fasting animals; the mean free sterol content of the mucosa was virtually identical in both groups.

The free sterol content of the upper third of the small intestine was higher than in the lower third. A paired *t* test revealed that this difference was statistically significant in both fed ($P < 0.01$) and fasting ($P < 0.025$) animals. In five rats the free sterol content of the middle third of the small intestine was also measured; the levels approximated those in the upper third. A paired *t* test did not demonstrate a significant difference between the esterified sterol content of the upper and lower intestine, although the mean was higher in the upper intestine. However, the quantities of esterified sterol were very small and the accuracy was correspondingly low.

TABLE 1 QUANTITIES OF FREE AND ESTERIFIED STEROLS IN THE SMALL INTESTINAL MUCOSA OF THE RAT, GUINEA PIG, AND RABBIT

Animal	Number of Animals	Mean DPS Concentration			
		Free Sterol		Esterified Sterol	
		Upper 1/3	Lower 1/3	Upper 1/3	Lower 1/3
		μmoles/g (wet wt)			
Rat (fed)	11	6.93*	6.44*	0.22	0.14
Rat (fasted)	11	6.98*	6.39*	0.32	0.23
Guinea pig	4	5.95	5.47	0.22	0.32
Rabbit	4	6.73	5.66	0.13	0.12

* A paired *t* test revealed a significant difference ($P < 0.025$) between the sterol concentration of the upper and lower parts of the intestine.

In the guinea pig and rabbit the distribution of free sterol was similar to that found in the rat. In all of the animals studied the total free sterol concentration in the mucosa of the upper small intestine was greater than that of the lower intestine. As in the rat, the content of sterol in the middle third of the intestine (measured in two guinea pigs and all four rabbits) was similar to that found in the upper third.

Composition. The relative proportions of different digitonin-precipitable free sterols in the small intestinal mucosa are presented in Table 2. Confirmation of the composition of the majority of the peaks was carried out with GLC–MS.

(*a*) *Rat* (*Fig. 3*). Cholesterol (peak 1) was the major component (88–96%) in all samples of mucosal free sterols. Although cholestanol had almost the same retention time on OV-17, mass spectrometry showed that it was not a significant constituent of the peak. The second peak had the retention time of lathosterol; GLC–MS confirmed that it was the major component. Lines were found at 351, 369, and 400 which are not present in the lathosterol spectrum. The presence of 7-dehydrocholesterol could account for the line at 351, but the other two lines are unexplained. The second peak formed 1.9–9.0% of the total area of the sterol chromatogram, and its relative area was constantly greater in the lower part of the small intestine than in the upper part (Table 2).

Peaks 3 and 4 in the rat were small, and usually peak 3 was the larger of the two; mass spectral analysis confirmed the presence of campesterol and β-sitosterol, respectively, as the major components. In the three rats fed rabbit chow for 2 wk prior to being killed, there was no increase in the relative proportion of plant sterols present in the mucosa.

(*b*) *Guinea Pig* (*Fig. 4*). The chromatographic patterns of the free sterols of the guinea pig mucosa were strikingly different from those found in the rat. In the upper small intestine the cholesterol peak (peak 1) occupied only 55–62% of the total area. Peak 2 occupied 19–25% of the total area, and its mass spectral pattern was compatible with a mixture of lathosterol and 7-dehydrocholesterol. The remainder (14–22%) of the chromatogram represented digitonin-precipitable materials found in the diet; peaks 3 and 4 were the largest and mass spectral patterns taken at their apices were almost identical with those of pure campesterol and β-sitosterol, respectively. The campesterol peak was similar in size to that due to β-sitosterol. The fifth

TABLE 2 RELATIVE AREAS OF THE PEAKS IN THE GAS–LIQUID CHROMATOGRAM OF THE MUCOSAL FREE STEROLS OF THE SMALL INTESTINE OF THE RAT, GUINEA PIG, AND RABBIT

Animals	Number of Animals	1*		2		3		4		5		6	
		U†	L†	U	L	U	L	U	L	U	L	U	L
		mean relative peak area‡											
Rat (fed)	4	93.9	88.9	2.3	6.4	2.9	3.4	1.0	1.4	—	—	—	—
Rat (fasted)	5	93.7	90.5	2.7	6.4	2.3	2.4	1.3	1.0	—	—	—	—
Guinea pig	4	57.9	79.8	23.6	14.8	7.0	4.2	7.6	1.3	3.2	—	<1.0	—
Rabbit §	4	89.0	91.0	2.1	3.3	5.0	3.3	3.9	2.4	—	—	—	—

* The numbers 1–6 refer to the peaks on the chromatograms shown in Fig. 3 (rat) and Fig. 4 (guinea pig).
† U, upper third of small intestine; L, lower third of small intestine.
‡ Percentage of total sterol area of the chromatogram.
§ In the rat and guinea pig the pattern of the sterols of the middle third of the intestine was virtually identical with that found in the upper third. In the rabbit, however, the plant sterol content was greater in the middle third (peak 1, 84.4%; 2, 2.2; 3, 7.6; 4, 5.8).

and sixth peaks were smaller; their mass spectral patterns, which have already been discussed, were identical with those of compounds with the same retention time found in the diet.

In the lower intestine of the guinea pig the pattern differed from that found in the jejunum. The cholesterol peak formed 79–81% of the total area. The second peak was broader and comprised 13–16% of the total, while the other peaks were much smaller than in the upper intestine and totaled only 4–8%. The third peak was the most prominent of these, but the relative retention time of the major component was longer than that of campesterol and corresponded with the retention times of stigmasterol and methostenol. Mass spectral characterization of this compound was not carried out. A small fourth peak, with the retention time of β-sitosterol, was observed, but the fifth and sixth peaks seen in the upper intestine were not discernible in the lower intestine. The pattern of sterols in the middle third of the guinea pig intestine was very similar to the pattern seen in the upper third.

(*c*) *Rabbit.* In the upper intestine of the rabbit the sterol pattern was similar to that in the rat, but the plant sterol peaks, campesterol and β-sitosterol, were more prominent and formed 5–11% of the area of the total sterol chromatogram. In the middle third of the intestine the plant sterol peaks were even more pronounced and made up 12–15% of the total sterols; in the lower intestine the plant sterols, although easily discernible, fell again to much lower levels. The campesterol peak was usually larger than the peak due to β-sitosterol. The lathosterol content of the mucosa was relatively low (1.7–4.1%). In three of the animals the relative proportion of lathosterol was higher in the lower than in the upper intestine, but the difference was less striking than in the rat.

Luminal Sterols of the Rat Intestine

Content and Concentration. In nine groups of rats (each group containing two or three animals) the contents of the upper, middle, or lower parts of the small intestine were pooled and ultracentrifuged. In all nine groups the total free sterol content of the aqueous phase was

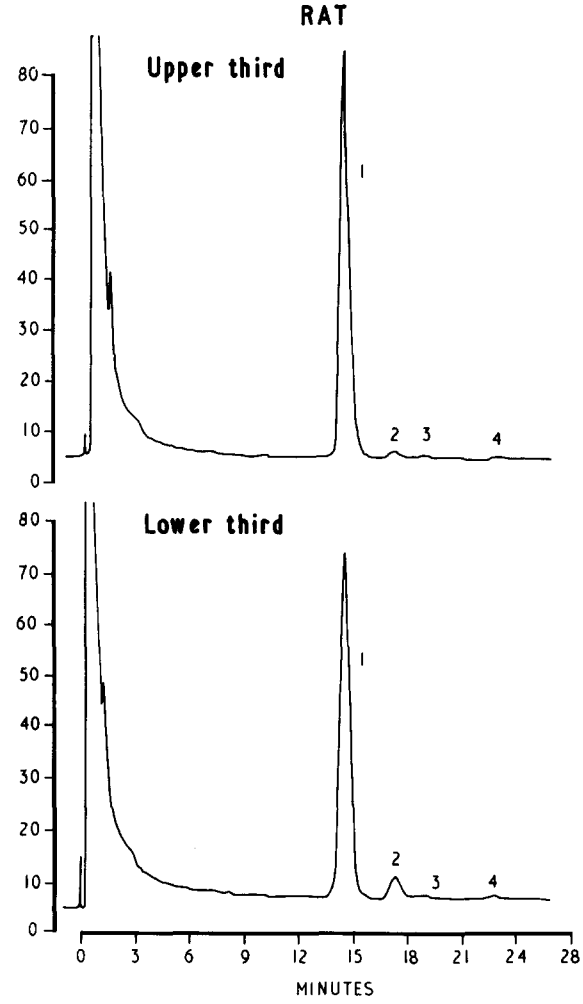


FIG. 3. Gas–liquid chromatograms of the digitonin-precipitable free sterols of the mucosa of the rat small intestine. Details of chromatographic technique are given in the text. Peak *1* is cholesterol; *2*, lathosterol; *3*, campesterol; and *4*, β-sitosterol.

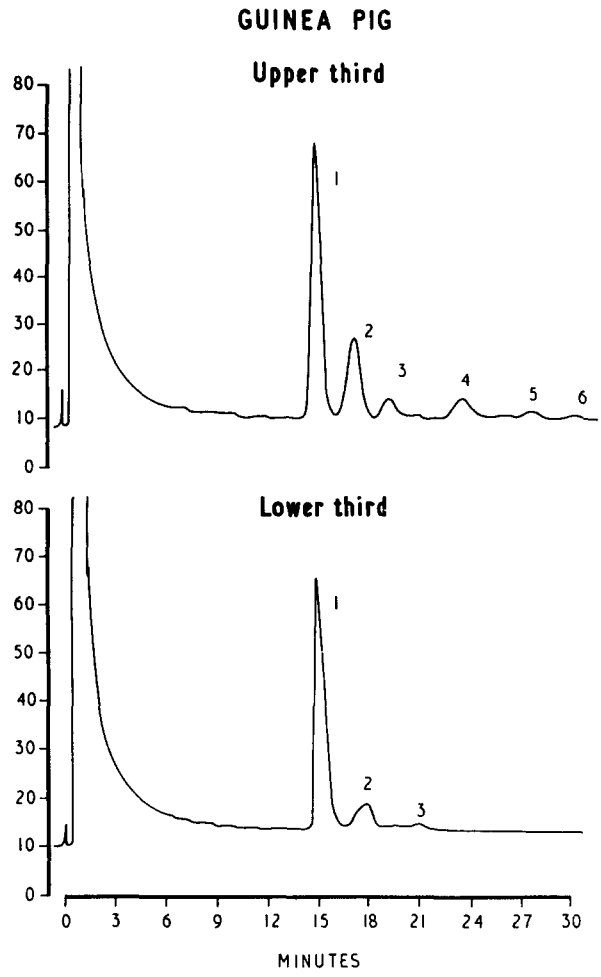


FIG. 4. Gas–liquid chromatograms of the digitonin-precipitable free sterols of the mucosa of the guinea pig small intestine. Details of chromatographic technique are presented in the text. Peak *1* is cholesterol; *2*, a mixture of lathosterol and 7-dehydrocholesterol; *4*, β-sitosterol; and *5* and *6*, unidentified compounds. Peak *3* in the upper third is due mainly to campesterol. In the lower third the retention time is longer than that of campesterol and would correspond to stigmasterol or methostenol; GLC–MS identification was not performed.

measured, and in four of these groups the sterol content of the sediment was also studied. The results (expressed as means) are presented in Fig. 5. The content of free sterols in the aqueous phase was higher in the upper and middle thirds of the intestine than in the lower third. Although the micellar free sterol content fell in the passage from the jejunum to the ileum, the sediment sterol increased markedly; the quantity appeared to correlate roughly with the amount of sediment visible after ultracentrifugation. Sedimentable sterol was markedly in excess of the sterol content of the aqueous phase at all levels of the small intestine, a relationship which was particularly striking in the lower gut.

In six groups of animals the *concentration* of free sterol was measured, and the results are presented in

TABLE 3 CONCENTRATION OF FREE DPS IN THE AQUEOUS PHASE OF THE LUMINAL CONTENTS OF THE RAT SMALL INTESTINE

Group	Number of Animals	Concentration of DPS	
		Upper* Intestine	Lower* Intestine
		nmoles/ml	
1	3	520	31
2	3	1200	200
3	2	465	25
4	2	260	35
5	3	890	150
6	3	920	165

* In groups 1–4 the intestinal contents from the upper and lower thirds of the small intestine were separated into aqueous phase and sediment. In groups 5 and 6 (which were also used for a separate study) the intestine was divided into upper and lower halves.

Table 3. Although there was considerable variation between individual groups, it is clear that the micellar sterol concentration of the upper intestine was very much higher than that present in the lower bowel. This accounted for the high micellar sterol content in the upper intestine, as the volume of aqueous phase present after ultracentrifugation was greater when lower intestinal contents were taken.

Composition. In six groups of animals the free sterols of the aqueous phase of the luminal contents were analyzed by GLC, and the sterols of the sediment were also examined in four of these groups. The results are presented in Table 4. The chromatographic outline of the plant sterol peaks in both the aqueous phase and the sediment was virtually identical with that found for dietary sterols. Their total area as plant

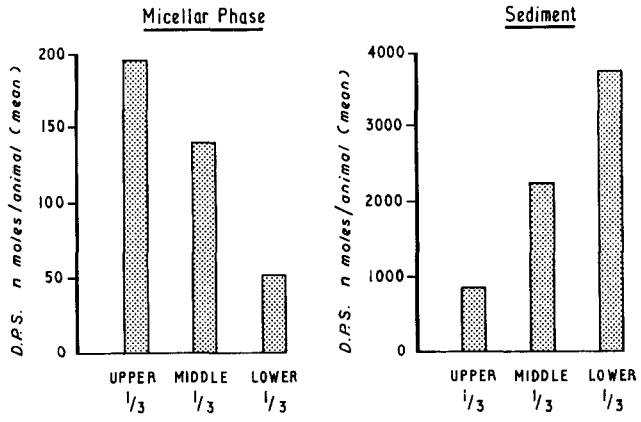


FIG. 5. The luminal content of micellar and sediment sterol at different levels of the rat small intestine. The results are expressed as the mean sterol content per animal. Only the free sterols were measured in the micellar phase and the results are mean values from 24 animals. Sediment sterols were saponified before assay and the results are means of the total sterol content (i.e., free plus esterified) from 12 animals.

TABLE 4 COMPOSITION OF THE STEROL CONTENT OF THE MICELLAR PHASE AND SEDIMENT OF THE LUMINAL CONTENTS OF THE RAT SMALL INTESTINE

Group	Fraction Analyzed	Cholesterol*			Lathosterol*			Plant Sterols*			Cholesterol:Plant Sterol Ratio		
		Upper	Mid	Lower	Upper	Mid	Lower	Upper	Mid	Lower	Upper	Mid	Lower
1	Micellar† phase	64.6	43.7	46.5	6.0	4.8	5.8	29.4	51.6	47.6	2.20	0.85	0.98
2		68.8	52.0	44.5	6.3	6.3	5.6	25.0	41.7	49.8	2.75	1.25	0.89
3		65.8	48.0	49.1	6.1	7.3	3.9	28.1	44.7	47.0	2.34	1.07	1.05
4		68.3	53.6	49.3	7.0	5.8	6.2	24.7	40.7	44.4	2.77	1.32	1.11
5‡		59.5		51.3	7.6		9.6	32.9		39.1	1.81		1.31
6‡		71.6		52.0	7.4		9.0	21.1		39.0	3.39		1.33
1	Sediment†	59.3	41.9	37.2	6.8	5.3	8.3	34.0	52.7	54.5	1.74	0.80	0.68
2		62.0	45.7	40.2	5.4	6.6	5.6	32.6	47.7	54.2	1.90	0.96	0.74
3		50.6	42.1	42.4	7.5	7.3	6.0	41.8	50.6	51.6	1.21	0.83	0.82
4		61.9	44.4	39.0	7.9	7.2	5.6	30.2	48.4	55.4	2.05	0.92	0.70

* Percentage of total sterol area of the chromatogram.
† Only the free sterol content of the micelles was analyzed, but the total sterol content of the sediment was used for GLC.
‡ In groups 5 and 6 the intestine was divided into upper and lower halves.

sterols is expressed as a percentage of the total area of the chromatograms. It is possible that sterols of animal origin such as methostenol and lanosterol might have been present, but if so they would have been only minor components.

There were several noteworthy results. First, the proportion of cholesterol in both the aqueous phase and sediment was much higher in the upper intestine than in the lower intestine, and in both areas the cholesterol content was higher than that which was present in rat chow. Second, there was reasonably good agreement between the sterol composition of the micelles and that of the sediment taken from the same part of the intestinal lumen, except that the proportion of cholesterol in the micelles was a little higher than that found in the sediment. As only free sterols were analyzed from the micellar phase and total sterols were examined from the sediment, this could have been explained if the proportion of cholesterol in the free sterols of the sediment were higher than that present in a quantitatively important ester fraction. Such a relationship was true of the dietary sterol content. In a separate study on four animals the sediment of the lower half of the intestine was taken, and the free and esterified sterols were measured. In each case the free sterol content was 16–21 times as great as the ester content. GLC revealed that the cholesterol content of the free fraction was indeed higher than that of the esterified fraction, but the striking preponderance of the free sterol fraction suggests that this could not explain the relative enrichment of micelles with cholesterol, which occurred both in the lower and upper intestine.

Finally, in all segments of the intestine there was a peak with the retention time of lathosterol; GLC–MS revealed a fragmentation pattern compatible with the presence of lathosterol as the major component, but with lines at 351, 369, and 400 similar to those found in the same peak when the mucosal sterols were analyzed. This peak constituted about 6–7% of the total sterols in both the micelles and the sediment. Although its content expressed as a percentage of the total sterol area was relatively constant from the upper to the lower intestine, it is clear that when it was expressed in relation to the plant sterols the concentration fell with passage along the intestine.

Sterol Composition of Shed Epithelium

In six animals GLC was performed on the epithelial debris shed into isolated loops of either jejunum or ileum. The results are presented in Table 5. The pattern of the sterols is similar to that seen in the mucosa of the normal animals (see Table 2).

DISCUSSION

It is clear from these studies that sterols other than cholesterol are found in significant amounts in the small intestinal mucosa of animals consuming a normal laboratory diet. Two types of noncholesterol sterol were found: plant sterols which are derived from the diet, and animal sterols, especially lathosterol, which are biosynthetic precursors of cholesterol. The noncholesterol sterol content was different in the rat, guinea pig, and rabbit, and there were marked variations

TABLE 5 STEROL COMPOSITION OF THE EPITHELIAL DEBRIS SHED INTO THE LUMEN OF SMALL INTESTINAL LOOPS

Origin of Debris	Cholesterol*	Lathosterol*	"Campesterol"*	"β-Sitosterol"*
Jejunum	95.0	2.2	2.3	<1.0
Ileum	93.4	3.7	2.4	<1.0

* Results are expressed as percentage of total sterol area of the chromatogram. They represent mean values of three animals in each group.

in the sterol pattern in different parts of the small intestine in the same animal.

The finding of plant sterols in the small intestinal mucosa of the species studied was not unexpected; several workers have shown that plant sterols can be taken up into the small intestinal mucosa of the rat (2–4) and fowl (6). However, it was surprising to find that the amount of plant sterol varied so widely between the three species; the rat intestine contained much less than either the guinea pig or rabbit. This difference was not purely dietary; when rats were fed for 2 wk on the same chow as guinea pigs and rabbits, there was little or no change in the plant sterol content of the rat mucosa. The reason for this difference is not clear, but it is possible that plant sterols are not taken up into the mucosa as readily in the rat as in the other two animals.

Our data suggest that the mucosa may be capable of discrimination in the uptake of individual sterols from the lumen. It is clear that in all three species the intestinal mucosa contains disproportionately more campesterol than β-sitosterol when the relative quantities of these substances are compared with those present in the diet, a result similar to that found by Boorman and Fisher in the fowl (6). If one may extrapolate from our results on intraluminal sterols in the rat, this observation cannot be explained on the basis of a greater micellar solubility of campesterol, since the relative proportions of campesterol and β-sitosterol in the micellar phase were the same as those found in the intestinal sediment and in the diet. Furthermore, the relatively low level of β-sitosterol in the mucosa cannot be explained by postulating that it is transferred more rapidly than campesterol into the intestinal lymph. There is evidence in the dog and fowl to suggest that lymphatic transport of campesterol is more efficient than that of β-sitosterol (5, 6), and GLC examination of the liver sterols of our guinea pigs revealed a campesterol peak which, although small, was appreciably larger than the corresponding peak due to β-sitosterol.[1] It would appear likely that the discrimination against β-sitosterol must be at the stage of its incorporation into the lipids of the mucosal cell, either at its luminal surface or at the stage of transfer to the deeper parts of the cell.

In all three species, peaks due to the presence of plant sterols were found in chromatograms of sterols from the upper, middle, and lower thirds of the intestine. In the guinea pig the difference between the upper and lower intestine was striking, and the plant sterols which were prominent in the upper and mid intestine were almost undetectable in the lower third. Similarly, in the rabbit the plant sterols were less prominent in the lower than in the upper and mid intestine. The variation in size of the plant sterol peaks noted for the rabbit and guinea pig is presumably a reflection both of their abundance in the lumen in micellar form and the ease of uptake into the intestinal cell. These findings are of interest in relation to the major site of sterol absorption in the mammal. Byers, Friedman, and Gunning (19) and Buchwald and Gebhard (20) suggested that the lower intestine is the major site of cholesterol absorption, but Swell et al. (21) and Borgström (22) concluded that the upper small intestine is more important. Our results suggest that luminal sterols *can* be incorporated into the mucosa at all levels of the small intestine but that in the guinea pig and rabbit sterol uptake from the lumen is quantitatively more important in the upper two-thirds.

In the rat, analyses of luminal sterols at different levels support the concept that more cholesterol is absorbed in the upper than in the lower small intestine. Efficient uptake of cholesterol from the lumen is dependent on its presence in a micellar phase (23). In our study the concentration of micellar sterols was far higher in the upper than in the lower intestine, and cholesterol itself made up a greater proportion of the total sterols; both of these factors would presumably enhance its absorption. Furthermore, with passage along the intestine the cholesterol:plant sterol ratio fell (Table 4). As plant sterols are poorly absorbed (3), this fall indicates cholesterol absorption (24). The change in the proportion of cholesterol to plant sterols was much greater between the upper and mid intestine than between the lower and middle thirds. Although such data must be interpreted with caution, they support the hypothesis that the upper small intestine is the major site for cholesterol absorption. Our finding that the mucosal free sterol concentration in the upper third of the intestine was significantly higher than in the lower third would also support such a regional difference in absorption.

Examination of the ultracentrifuged small intestinal contents from the rat revealed that only a small proportion of the luminal sterols was found in the aqueous supernatant, while the remainder was present in the sediment. In the upper third of the small bowel the aqueous phase contained approximately 20% of the luminal sterols, but in the lower third only 1–2% of the available sterols were in micellar form. GLC of both sediment and supernatant revealed that, though the relative proportions of different sterols varied considerably with passage along the intestine, there was good agreement at each level between the sterol patterns of supernatant and sediment. It is possible that equilibration between the sediment and supernatant may have occurred while the intestinal contents were standing

[1] McIntyre, N., and K. Kirsch. Unpublished observations.

prior to ultracentrifugation; even so, it would appear that the sterols of the sediment are available for incorporation into a micellar phase, and thus for absorption.

The large amount of sterol found in the sediment is of interest. In most studies relating to the absorption of sterols, it has been assumed that luminal sterols are distributed between a micellar phase and an oil phase (25–27). This concept has been derived from studies in which aspiration of intestinal contents has been performed after introduction of relatively large amounts of fat into the lumen (25). In the laboratory diet fed to our rats there was little fat, and visual examination of the surface of the supernatant after ultracentrifugation revealed no evidence of a supranatant emulsion; furthermore, chemical analysis revealed no evidence for a cholesterol-rich surface layer. Simmonds, Hofmann, and Theodor (28) found that during perfusion of the small intestine in man, precipitation of cholesterol occurred as fatty acid and monoglyceride were absorbed from mixed micelles. We agree with their view that "any complete description of the intraluminal phase of sterol absorption should probably consider a sediment phase as well as an oil and micellar phase." Our results suggest that the role of the oil phase may have been overemphasized; when the diet is relatively low in fat the oil phase may be unimportant and micellar sterols may be derived directly from the sediment.

In the rat, guinea pig, and rabbit, noncholesterol sterol of endogenous origin was found in the small intestinal mucosa. In the rat and rabbit only lathosterol was easily detectable by the methods used; in the guinea pig the presence of appreciable amounts of 7-dehydrocholesterol was confirmed by GLC–MS. In the rat, lathosterol was also present in the luminal contents. Wells, Coleman, and Baumann (7) identified lathosterol in large amounts in the feces of rats on a fat-free diet and also found it in the small intestinal mucosa. As this sterol was not present in the diet or in rat bile, Wells and his colleagues concluded that the small intestinal mucosa was a major source of fecal lathosterol. However, they calculated that, to account for the lathosterol excreted, the whole mucosa would have to be shed and renewed in 12 hr, a time much shorter than the current estimates of epithelial cell turnover time (29, 30).

The present data provide evidence suggesting that the small intestinal mucosa is not the major source of fecal lathosterol. When epithelial debris was collected from isolated loops of jejunum and ileum, the proportion of lathosterol in DPS was lower than that found in the lumen of the intact animal. Furthermore, lathosterol was present in the lumen in large amounts at all levels of the small intestine, and it was also found in the stomach contents.[1] The source of the luminal lathosterol cannot be stated with certainty, but most likely it was derived from licking of the fur, since lathosterol is a major sterol of rat skin (15, 31, 32). Kellogg and Wostmann (33) have suggested that only small amounts of cholesterol and its precursors would be ingested by skin grooming and that they would be almost totally absorbed; however, Miettinen's unpublished study (cited by Gustafsson, Gustafsson, and Sjövall [34]) showed that when rats were prevented from licking their fur, there was a pronounced decrease in fecal lathosterol.

Cholesterol is produced in large amounts in the small intestinal mucosa (35), and lathosterol is a precursor at the end of the biosynthetic pathway (36). The presence of lathosterol in the small intestinal mucosa could therefore be explained either by absorption from the lumen or by local synthesis. In the rat the mucosal lathosterol content is highest in the lower third of the small intestine, where cholesterol synthesis is maximal (35) and sterol absorption probably minimal. Furthermore, both biliary diversion and obstruction, which abolish sterol absorption and increase mucosal cholesterol synthesis, are associated with a rise in the lathosterol content of the mucosa at all levels of the small intestine.[1] Thus, in the rat it would appear that mucosal lathosterol is largely the result of local synthesis. In the guinea pig the situation is more complicated, but other studies suggest that in this species much of the mucosal lathosterol may have been absorbed from the lumen.[1]

We would conclude that the analysis of noncholesterol sterols in the mucosa and lumen of the small intestine may provide evidence not only on the source of intraluminal sterols, but also on the site of their absorption. As noncholesterol sterols form a significant proportion of the small intestinal sterols, their presence clearly cannot be ignored. In the past there were no satisfactory methods for the study of sterol mixtures, but with the introduction of newer techniques the measurement of noncholesterol sterols can prove of value in the interpretation of the complex role of the small intestine in the metabolism of cholesterol and other sterols.

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