Biosynthesis of Δ^7-cholesten-3β-ol, $\Delta^{5,7}$-cholestadien-3β-ol, and Δ^5-cholesten-3β-ol by guinea pig intestinal mucosa in vitro

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ABSTRACT Methods were developed for the separation and determination of the various 27-carbon sterols of intestinal mucosa by means of thin-layer chromatography. Scrapings of the mucosa of the small intestine of guinea pig and rat were shown to incorporate isotope from ^{14}C-labeled acetate and mevalonate into sterols in vitro. For each substrate this activity was lowest in mucosa from the proximal third of the small intestine and greatest in mucosa from the more distal regions of the small intestine.

The total 27-carbon sterol *content* of guinea pig mucosa varied only slightly along the length of the small intestine, but the concentration of cholesterol was highest distally. More than 95% of the radioactivity incorporated from acetate-2-^{14}C into 27-carbon sterols by guinea pig mucosa in 4 hr was recovered as lathosterol and 7-dehydrocholesterol; less than 5% was in cholesterol. The specific activities of the 27-carbon sterols were consistent with the concept that synthesis proceeds from lathosterol to 7-dehydrocholesterol to cholesterol.

KEY WORDS acetate · mevalonate · incorporation · 27-carbon sterols · cholesterol · 7-dehydrocholesterol · lathosterol · guinea pig · rat · small intestine mucosa · proximal · distal · sterol biosynthesis

A NUMBER OF tissues other than liver can synthesize cholesterol de novo from such simple precursors as acetate. In 1950, Srere, Chaikoff, Treitman, and Burstein (1) showed by measurement of incorporation of acetate-^{14}C into digitonin-precipitable material in vitro that slices of small intestine were nearly as active as liver slices. At about the same time, Popják and Beeckmans (2) found that various tissues of intact, pregnant rabbits incorporated both deuterated water and acetate-^{14}C into digitonin-precipitable sterols. The sterols in intestine were labeled more rapidly and attained a higher specific activity than sterols of any other tissue studied, and the authors concluded that in the rabbit the intestine was the most active site of sterol biosynthesis. Gould, Taylor, Hagerman, Warner, and Campbell (3) showed during studies of the effect of dietary cholesterol on its endogenous synthesis that the intestinal mucosa of dog can incorporate acetate-^{14}C into digitonin-precipitable material. In 1955, Schwenk, Alexander, and Fish (4) found that after parenteral administration of acetate-^{14}C to guinea pigs, the sterols of the intestine and of its contents that precipitated with digitonin but did not form insoluble dibromides were labeled to a greater extent than other sterols of intestine or liver. In a brief communication Mercer and Glover (5) reported that everted sacs of guinea pig small intestine incorporated acetate-^{14}C and mevalonate-^{14}C from the medium bathing the mucosa into various C_{27}-sterols including lathosterol, 7-dehydrocholesterol, and cholesterol.

Thus, there is abundant evidence to show that the mammalian small intestine is capable of de novo sterol biosynthesis. However, interpretation of results in most instances is limited because it is not possible to assess the influences of plasma and biliary sterols in the in vivo studies, and because tissue samples for the in vitro studies included all layers of intestinal wall, rather than mucosa alone. In the present report, experiments are described in which the incorporation of acetate-^{14}C and mevalonate-^{14}C into C_{27}-sterols by suspensions of scrapings of mucosa from the small intestine of guinea

Abbreviations: C_{27}-sterols, 27-carbon sterols; lathosterol, Δ^7-cholesten-3β-ol; 7-dehydrocholesterol, $\Delta^{5,7}$-cholestadien-3β-ol; cholesterol, Δ^5-cholesten-3β-ol; TLC, thin-layer chromatography.

pig and rat was determined. The individual sterols were isolated and assayed. The activities of different anatomical regions of the small intestine were compared. A brief report of this work was published previously (6). At the same time Dietschy and Siperstein published an abstract describing their studies of the incorporation of acetate-^{14}C into sterols by intestinal mucosa in vitro (7), and these data later appeared in a detailed publication (8).

MATERIALS

Sodium acetate-2-^{14}C and cholesterol-4-^{14}C were purchased from the Nuclear-Chicago Corp., Des Plaines, Ill. DL-Mevalonic acid-2-^{14}C was obtained as the dibenzyl ethylenediamine salt (Volk Radiochemical Co., Skokie, Ill.), dissolved in water, and hydrolyzed by addition of a slight excess of KOH and brief exposure to heat. After neutralization with HCl, the dibenzyl ethylenediamine was extracted with hexane. DL-Mevalonic acid lactone (Sigma Chemical Co., St. Louis, Mo.) was dissolved in water and converted to the open form by addition of a slight excess of KOH and neutralization with HCl. Commercial lathosterol and 7-dehydrocholesterol (Calbiochem, Los Angeles, Calif.) and cholesterol (Nutritional Biochemicals Corp., Cleveland, Ohio) were recrystallized from methanol.

Female Hartley strain albino guinea pigs, 300–500 g, and female Sprague-Dawley rats, 200–250 g, were maintained on ad lib. standard laboratory diets.

METHODS

Preparation of Tissue and Incubation

Unfasted animals were decapitated and the small intestine was immediately rinsed in situ with ice-cold Krebs-Ringer bicarbonate medium (9) modified to contain half the usual concentration of calcium ion and 5.6 mM glucose. The small intestine was excised, rinsed again, drained, and opened longitudinally on a chilled surface. The mucosa was blotted with filter paper, scraped from the submucosa and muscularis with a blunt spatula, and placed in a weighed 25 ml Erlenmeyer flask containing 2.5–3.0 ml of chilled medium and isotopically-labeled substrate. When the activity of one intestinal region was compared with that of another, the small intestine was divided appropriately before the mucosa was removed. The mucosal scrapings were suspended in incubation medium and disrupted by gentle agitation. The flask was flushed with 95% O_2–5% CO_2, stoppered, and weighed again to determine wet tissue weight. The mixture was incubated in a shaking incubator at 37°C for the specified time, usually 2 hr.

Liver slices were prepared with a Stadie-Riggs microtome; incubation procedures were the same as those for intestinal mucosa.

Saponification and Extraction

The reaction was stopped by addition of 2 ml of saturated KOH solution. The flask contents were transferred to a graduated 45 ml tube, the flask was rinsed twice with a total of 5 ml of absolute ethanol, and the washings were added to the tube. For liver slices, twice these volumes were used. The tube was flushed with N_2, stoppered, and incubated at 60°C for 2 hr with frequent agitation. The ethanol was evaporated under N_2, 2 ml of 100% ethanol was added, and the mixture volume was brought to 10 ml with distilled water.

The nonsaponifiable material was extracted 3 times with 10 ml of hexane. The pooled extract was washed 4 times with 0.1 M sodium acetate solution and twice with distilled water, and dried over anhydrous sodium sulfate. It was reduced in volume to about 2 ml under a stream of N_2 and it was used for chromatography, for digitonide formation, or for both.

Isolation of Sterols from Nonsaponifiable Material

The following TLC systems described by Avigan, Goodman, and Steinberg (10) and modified as indicated were used.

C_{27}-Sterol Fraction (TLC system 1). Isolation of C_{27}-sterols as a group was carried out as described by Avigan et al. (10). The zone of C_{27}-sterols was scraped from the plate and the sterols were eluted with chloroform by the use of a glass aspirator (11). Aliquots of eluate were subjected to additional chromatography, to analysis for sterol content, to assay for radioactivity, or to combinations of these procedures. Aliquots for chromatography were dried under N_2 and the residue was dissolved in hexane.

7-Dehydrocholesterol (TLC system 2). Separation of 7-dehydrocholesterol from cholesterol and lathosterol (Fig. 1) was achieved by chromatography of the C_{27}-sterol fraction on TLC plates coated with Silica Gel G and impregnated with $AgNO_3$ (10). The solvent was benzene–ethyl acetate 3:1. The 7-dehydrocholesterol zone and the zone of remaining C_{27}-sterols were scraped from the plate and eluted with chloroform.

Lathosterol and Cholesterol (TLC system 3). The remaining C_{27}-sterols from TLC system 2 were separated by chromatography on 50-cm plates in benzene–ethyl acetate 20:1 for 48 hr (10). Cholesterol migrated approximately 1.2 times as far as lathosterol (Fig. 1).

Recovery of Sterols during Isolation Procedures

Factors were determined for correction of experimental results for incomplete recovery of sterols during isolation.

TABLE 1 RECOVERY OF STEROLS DURING ISOLATION PROCEDURE

Step in Isolation Procedure	Lathosterol		7-Dehydrocholesterol		Cholesterol	
	Recovery in Individual Step	Cumulative Recovery	Recovery in Individual Step	Cumulative Recovery	Recovery in Individual Step	Cumulative Recovery
	%		%		%	
1. Hexane extract of nonsaponifiable material	92	92	93	93	93	93
2. TLC system 1: C_{27}-sterols	91	84	91 (80)	80	95 (97)	90
3. TLC system 2	94	79	80 (84)	65	98 (95)	87
4. TLC system 3	73	57	—	—	80 (91)	74

The results in parentheses were obtained in an experiment in which step 1 was omitted and the labeled sterols were applied directly to the TLC plate for step 2. When duplicate determinations are available the average of the two values is used to calculate cumulative recovery.

For this purpose, biosynthetically labeled lathosterol and 7-dehydrocholesterol were prepared by incubation of guinea pig intestinal mucosa with acetate-2-^{14}C and subsequent isolation of the two sterols as described. Three standard incubation mixtures were prepared without labeled substrate and a known amount of radioactivity as 7-dehydrocholesterol-^{14}C, lathosterol-^{14}C, or cholesterol-4-^{14}C was added to each and reisolated. Recovery at each step in the isolation was determined separately for the three sterols (Table 1). In a second study, recoveries of 7-dehydrocholesterol-^{14}C and cholesterol-4-^{14}C were determined for the TLC steps only (Table 1). The cumulative recovery of C_{27}-sterols as a group (step 2) was taken as 85%, the average of the values for each of the sterols (84, 80, and 90%). This value and the final values for cumulative recovery of the individual sterols were used to correct all values given below for tissue concentration of sterols and for incorporation of precursor label into sterol. In no instance did this correction alter the qualitative relationships among the data.

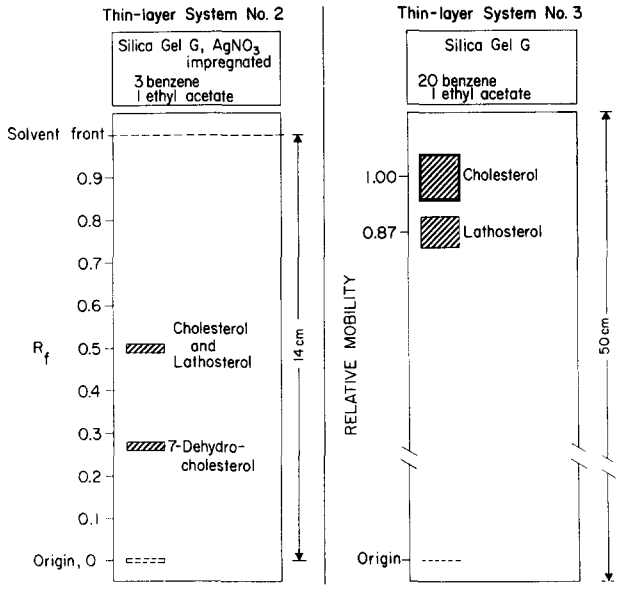

FIG. 1. TLC systems 2 and 3 for the isolation of individual C_{27}-sterols. Samples were dissolved in hexane and applied as bands at the origin. After the plates were developed in the indicated solvent systems, they were dried, sprayed with an alcoholic solution of Rhodamine 6G, and examined under ultraviolet light. All the sterol zones exhibited a yellowish-pink fluorescence, except for the 7-dehydrocholesterol zone which appeared purple.

Analytical Procedures

The Liebermann-Burchard reaction (12) was used to determine the sterol content of eluates from TLC plates. 7-Dehydrocholesterol was measured by means of its ultraviolet absorption, and irrelevant absorption was corrected for by the method of Festenstein and Morton (13). An extinction coefficient of 11,840 M^{-1} cm^{-1} at 281.5 mμ (14) was used for calculations. Specimens dissolved in absolute ethanol were placed in quartz cuvettes (1 cm light path) and their absorbancy was measured in a Zeiss PMQ-II spectrophotometer.

For determination of radioactivity of cholesterol in a mixture of ^{14}C-labeled C_{27}-sterols, the dried mixture was weighed and a measured amount of unlabeled cholesterol, approximately 100 times the weight of the mixture, was added. The dibromide of cholesterol was formed and debrominated (15). The cholesterol was reisolated and recrystallized twice. Its specific activity times the weight of carrier cholesterol added to the original mixture was taken as the amount of radioactivity present as cholesterol in the mixture.

Digitonides were formed from the nonsaponifiable material according to the method of Sperry and Webb (16). The washed and dried derivatives were dissolved in 1.0 ml of pyridine and cleaved by heating them at 80°C for 1 hr. The free sterols were then extracted into diethyl ether.

Radioactivity was determined in a Packard Tri-Carb liquid scintillation spectrometer. Either of two methods was used. In the one used most extensively, an aliquot of sterol solution was dried in a counting vial and assayed in 15 ml of 0.5% 2,5-diphenyloxazole in toluene. The other method was used in a few experiments and only to assay the C_{27}-sterol zone (TLC system 1). The zone was scraped from the chromatography plate directly into a counting vial and suspended in a mixture

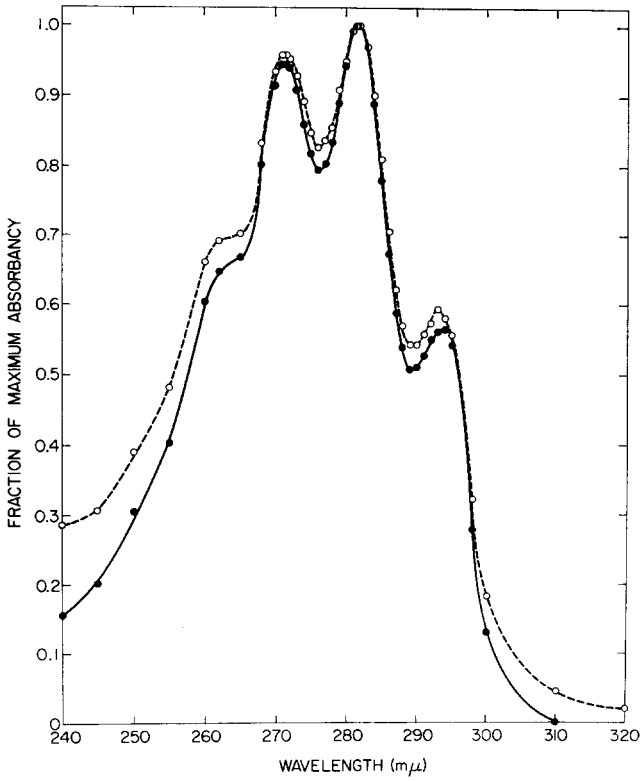

FIG. 2. Ultraviolet absorption spectra of isolated and authentic 7-dehydrocholesterol. The samples were dissolved in spectroscopically pure ethanol. For purposes of comparison absorbancy values are plotted as the fraction of the value for the maximum value at 281.5 mμ. Closed circles represent authentic 7-dehydrocholesterol, open circles the material isolated from guinea pig intestinal mucosa.

containing 0.5% 2,5-diphenyloxazole, 0.03% 1,4-bis-[2-(4-methyl-5-phenyl oxazolyl)]-benzene (dimethyl POPOP) and 4% thixotropic gel (Cab-O-Sil, Cabot Corp., Oxides Div., Boston, Mass.) in toluene (17). Radioactivity as counts per minute was converted to disintegrations per minute by means of the channels-ratio method (18). Counting efficiency was approximately 75% for each system.

RESULTS

Incorporation of Acetone-2-^{14}C into C_{27}-Sterols by the Mucosa: Guinea Pig

Whether isolated mucosal scrapings from the small intestine of the guinea pig incorporate acetate into C_{27}-sterols in vitro was tested by incubation of mucosa from the entire length of small intestine with acetate-2-^{14}C and subsequent isolation of the C_{27}-sterols. Not only was incorporation observed, but most of the radioactivity of the nonsaponifiable material was recovered as C_{27}-sterols. Of the radioactivity in the C_{27}-sterol fraction, 98% was isolated as lathosterol, 7-dehydrocholesterol, and cholesterol.

The identification of these sterols is based on the following considerations. The material designated lathosterol was nonsaponifiable lipid with the same mobility as authentic lathosterol in each of the three TLC systems. It was found to contain no (or <3%) slow-acting sterols when tested with the Liebermann-Burchard reagent. The presence of $\Delta^{7,24}$-cholestadien-3β-ol was not excluded. The material considered to be 7-dehydrocholesterol was nonsaponifiable and corresponded in mobility to 7-dehydrocholesterol in two TLC systems. The Liebermann-Burchard reaction revealed no more than 0.7% slow-acting sterols. The ultraviolet absorption spectrum of this material corresponded to that of the authentic compound (Fig. 2).

The material initially regarded as cholesterol (TLC system 3) was not saponifiable and it migrated with standard cholesterol in each of the three TLC systems. Analysis with the Liebermann-Burchard reagent, however, disclosed small, but probably significant, amounts of a fast-acting sterol (2.5 and 8.0% in two determinations). When this material was purified via the dibromide, only 50–75% of the radioactivity was found to be in cholesterol. Although the remaining radioactivity, which was not precipitated by bromine, accounted for only about 1–2% of the total radioactivity incorporated into C_{27}-sterols by intestinal mucosa, it nearly equaled that in cholesterol. Unless otherwise mentioned, all values given for radioactivity in, or for precursor incorporation into, cholesterol are based on material purified via the dibromide. The identity of the sterol or sterols contaminating the cholesterol band in TLC system 3 is not known. Slight contamination by the much more highly labeled lathosterol could explain the presence both of fast-acting sterol and of radioactivity in compounds not precipitable by bromine, but the presence of small amounts of other contaminants such as desmosterol ($\Delta^{5,24}$-cholestadien-3β-ol) and 5α-cholestan-3β-ol is not excluded.

The specific activities of the isolated individual sterols were determined in two experiments (Table 2). Despite variation between the results of the two experiments, in each case the specific activity of lathosterol was greater than that of 7-dehydrocholesterol, which in turn exceeded that of cholesterol.

Effect of Incubation Time on Acetate Incorporation

Because of the variability of results obtained with different animals, an example of which is shown in Table 2, the experimental design was modified for the study of the effect of incubation time on incorporation of isotope from acetate-2-^{14}C into C_{27}-sterols as a group and into cholesterol. The distal half of a guinea pig small intestine was opened longitudinally and divided lengthwise into two approximately equal strips. The mucosa was scraped

TABLE 2 SPECIFIC ACTIVITIES OF STEROLS OF GUINEA PIG INTESTINAL MUCOSA AFTER INCUBATION WITH ACETATE-2-^{14}C

Expt.	Specific Activity		
	Lathosterol	7-Dehydro-cholesterol	Cholesterol
		dpm/μg	
1	1132	372	2.61
2	153	57.2	5.07*

Mucosal scrapings from the entire length of small intestine were incubated 2 hr, standard conditions, 4.6 mM acetate-2-^{14}C, 2.88 × 10^7 dpm per flask, total volume 7.94 ml.

* Specific activity value indicated is based on total amount of radioactivity eluted from the band moving with cholesterol in TLC system 3. The true specific activity of cholesterol is therefore even lower than that given (see Results).

TABLE 3 EFFECT OF TIME ON INCORPORATION OF ACETATE-2-^{14}C INTO C_{27}-STEROLS AND CHOLESTEROL BY GUINEA PIG INTESTINAL MUCOSA

Expt.	Tissue Weight	Incubation Time	Acetate Incorporation		
			C_{27}-Sterols (*a*)	Cholesterol (*b*)	*b*/*a* × 100
	mg	*hr*	*mμmoles/g wet weight of mucosa*		%
3	238	1	413	4.27	1.03
	233	2	952	12.3	1.29
4	115	2	652	17.3	2.65
	122	4	1428	49.8	3.49

Results are for paired mucosal specimens. Standard incubation conditions, with 4.6 mM acetate containing 5.11 × 10^6 dpm per flask.

from each half and incubated in the usual manner. In this way paired incubations were prepared with mucosa derived from the same anatomical site of the same animal, and a given animal served as its own control. In one experiment the paired flasks were incubated for 1 and 2 hr and in another experiment for 2 and 4 hr. The C_{27}-sterols were isolated, assayed, and brominated to isolate cholesterol (Table 3). Although the values obtained for the 2 hr incubations differed for the two animals, the data indicate that incorporation of isotope into C_{27}-sterols is nearly linear for 4 hr. The fraction of the total amount of isotope in C_{27}-sterols that was in cholesterol increased slightly with time.

Sterol Content and Biosynthesis in Different Regions of Guinea Pig Small Intestine

Since many of the metabolic functions of the intestinal mucosa vary in activity from one region of the small intestine to another, sterol metabolism was studied in mucosal scrapings taken from the proximal, middle, and distal thirds of guinea pig small intestine (Table 4).

Values for concentrations of the three C_{27}-sterols in mucosa from the three intestinal regions were determined in experiments 5–7. Although there is variability among animals, the following generalizations can be made. The concentration of C_{27}-sterols as a group changes but little from one region of the intestine to another, but the value for the middle third of the mucosa is consistently higher than for the other segments. In all three intestinal regions cholesterol accounts for well over 50% of the total C_{27}-sterols; this value varies from 60% in the proximal third, to 65–69% in the middle third, to 80–82% in the distal third. The amount of lathosterol is markedly lower in the distal third. In the proximal third it accounts for 20–23% of the C_{27}-sterols; in the middle third, 17–19%; and in the distal third 4–7%. Similar, but less marked, changes are seen with 7-dehydrocholesterol. It accounts for 16–20% of C_{27}-sterols in the proximal third; 13–18% in the middle third; and 10–16% in the distal third. In each experiment its concentration is lowest in the distal third.

The incorporation of acetate-2-^{14}C into C_{27}-sterols as a group was determined in experiments 5–9. In no instance is the value for proximal intestine the greatest of the three; and in all but experiment 7, the incorporation is significantly greater in the distal two-thirds of the small intestine than in the proximal third. In experiment 7 the activities of the proximal and distal thirds are the same and that of the middle third is somewhat higher. Incorporation into the individual C_{27}-sterols was determined in experiments 5 and 6. Incorporation of acetate-2-^{14}C into lathosterol and 7-dehydrocholesterol is greater in the distal two-thirds of the small intestine than in the proximal third; incorporation into cholesterol (experiments 5–7) does not vary as much from one region of the intestine to another.

In experiment 8 an aliquot of the nonsaponifiable material was assayed for incorporation of acetate-2-^{14}C into digitonin-precipitable material. The values for proximal, middle, and distal mucosa were 540, 748, and 635 mμmoles/g and are to be compared to 427, 678, and 594 mμmoles/g respectively (Table 4), the values determined for the C_{27}-sterol fraction from TLC system 1.

In a single study (experiment 9) the effect of a fourfold increase in acetate concentration was examined. Again the activity of the distal two-thirds of the small intestine exceeds that of the proximal third; no stimulation of activity is apparent; and it is doubtful whether the results vary significantly from the range of values observed with the lower concentration of acetate.

In the two experiments, 5 and 6, in which values for specific activity of the sterols were determined, the results are similar to those of experiments 1 and 2 (Table 2). The specific activity of lathosterol is greater than that of 7-dehydrocholesterol, which exceeds that of cholesterol. The only exception is the result for the distal third of small intestine, experiment 5, in which the specific activity of lathosterol equals that of 7-dehydrocholesterol, but both greatly exceed that of cholesterol.

TABLE 4 COMPARISONS OF INCORPORATION OF ACETATE-2-^{14}C INTO STEROLS BY MUCOSAL SCRAPINGS FROM PROXIMAL, MIDDLE, AND DISTAL THIRDS OF GUINEA PIG SMALL INTESTINE AND BY LIVER SLICES

Expt.	Tissue Source (Intestinal Third or Liver)	C_{27}-Sterols		Lathosterol			7-Dehydrocholesterol			Cholesterol		
		Concentration	Acetate Incorporation	Concentration	Acetate Incorporation	Specific Activity	Concentration	Acetate Incorporation	Specific Activity	Concentration	Acetate Incorporation	Specific Activity
		μmoles/g	*mμmoles/g*	*μmoles/g*	*mμmoles/g*	*dpm/μg*	*μmoles/g*	*mμmoles/g*	*dpm/μg*	*μmoles/g*	*mμmoles/g*	*dpm/μg*
5	Proximal	6.69	342	1.55	226	190	1.09	103	123	4.05	12.7	4.06
	Middle	7.98	521	1.45	371	329	1.01	138	175	5.52	12.3	2.87
	Distal	7.12	589	0.49	347	365	0.80	229	367	5.83	12.9	2.87
	Liver	5.57	29.6	0.05	8.3	72.4	—	—	—	5.52	20.7	4.85
6	Proximal	5.39	86.3	1.10	53.9	67.9	1.04	30	41.1	3.25	2.35	1.32
	Middle	5.45	168	1.03	112	154	0.78	53.1	97.4	3.64	2.67	1.80
	Distal	5.26	252	0.39	139	495	0.54	108	278	4.33	5.29	3.28
	Liver	3.38	70.3	0.05	10.1	—	—	—	—	3.33	58.6	26.3
7	Proximal	6.12	610	1.29	—	—	1.22	—	—	3.61	10.6	—
	Middle	6.62	703	1.14	—	—	1.17	—	—	4.31	9.81	—
	Distal	6.25	614	0.26	—	—	1.01	—	—	4.98	9.81	—
	Liver	5.18	89.1	0.05	—	—	—	—	—	5.13	51.9	—
8	Proximal	—	427									
	Middle	—	678									
	Distal	—	594									
9	Proximal	—	181									
	Middle	—	591									
	Distal	—	270									

Incubations: 2 hr; volume: 2.75 ml in experiments 6 and 8, 3.0 ml in others. Cholesterol isolation included precipitation as the dibromide. Tissue slices used in experiments with liver. Acetate 4.7 mM in experiments 5–8, 17.1 mM in experiment 9, radioactivity 6.2–10 × 10^6 dpm per flask. In experiments 5–7 values given for C_{27}-sterols are the sum of corresponding values for individual sterols, when determined; otherwise values for C_{27}-sterols were determined directly. Acetate incorporation expressed per gram of wet tissue.

TABLE 5 INCORPORATION OF MEVALONATE-2-^{14}C INTO C_{27}-STEROLS IN VITRO BY GUINEA PIG MUCOSAL SCRAPINGS AND LIVER SLICES

Expt.	Incorporation of Mevalonate-2-^{14}C into C_{27}-Sterols			
	Mucosal Scrapings from Small Intestine			
	Proximal Third	Middle Third	Distal Third	Liver Slices
	mμmoles/g wet tissue			
10	409	1827	1035	293
11	728	780	838	176
12	1010	1500	1230	

Incubations, 2 hr, substrate concentration 5 mM DL-mevalonate-2-^{14}C, 4.2–16 × 10^5 dpm per flask, volume of incubation mixture 3.0 ml.

Comparison of Intestinal Mucosa to Liver

In experiments 5–7 (Table 4) the studies were extended to include liver slices. The concentration of total C_{27}-sterols in liver tissue is slightly lower than it is in mucosal scrapings. The liver C_{27}-sterols are 99% cholesterol and 1% lathosterol. No 7-dehydrocholesterol is detected. The incorporation of acetate-2-^{14}C into C_{27}-sterols is consistently lower for liver slices than for mucosal scrapings. However, about two-thirds of the isotope incorporated into C_{27}-sterols by liver slices is isolated as cholesterol. Thus, incorporation of precursor into cholesterol is relatively and absolutely greater for liver slices than for mucosal scrapings.

Incorporation of Mevalonate into C_{27}-Sterols by Mucosal Scrapings and Liver Slices

To determine whether the differences observed for incorporation of acetate-2-^{14}C into C_{27}-sterols among various regions of the small intestine, and between intestine and liver, apply also to the formation of C_{27}-sterols from mevalonate, we performed experiments 10–12 (Table 5). The incorporation of mevalonate exceeds that of acetate for the middle and distal thirds of the small intestine and for the liver slices. As with acetate, incorporation of mevalonate is significantly greater in the distal two-thirds of the small intestine than in the proximal third, and the activity of the mucosal scrapings exceeds that of the liver slices.

Incorporation of Acetate and Mevalonate into C_{27}-Sterols by the Mucosa: Rat

Incorporation of labeled acetate and mevalonate into C_{27}-sterols by mucosal scrapings from the proximal, middle, and distal thirds of the small intestine of the rat was studied (Table 6). The activity of rat mucosa is significantly lower than that of guinea pig in all experiments except 13b and 13i. In these experiments the mucosa from the distal third is as active as some of the guinea pig specimens. As in the case of the guinea pig, mucosa from the proximal third of the rat small intestine is least active, but in the rat the activity is much more sharply localized to the distal third of the small intestine.

TABLE 6 INCORPORATION OF ACETATE-2-^{14}C AND MEVALONATE-2-^{14}C INTO C_{27}-STEROLS IN VITRO BY MUCOSAL SCRAPINGS FROM RAT SMALL INTESTINE

Expt.	Substrate	Incorporation of Substrate into C_{27}-Sterols: Mucosal Scrapings from Small Intestine — Proximal Third	Middle Third	Distal Third
		mμmoles/g wet tissue		
13a	Acetate	0.61	0.47	2.24
b		17.8	4.17	227
c		0.29	1.50	60.9
d		0.77	0.12	2.53
e		0.33	5.48	7.15
f		0.32	0.94	10.5
g		1.38	3.30	1.28
h		0.48	1.21	1.43
i		1.48	2.12	102
14	Mevalonate	4.17	6.70	66.3

Incubations 2 hr, substrate concentration experiment 13, 17.1 mM acetate-2-^{14}C, 10^5 dpm per flask, experiment 14, 16.9 mM mevalonate-2-^{14}C, 1.8×10^6 dpm per flask, volume of incubation mixture 3.0 ml.

There is much greater variation among rats than among guinea pigs. Incorporation of mevalonate is much greater in the distal third of the small intestine than in the other two-thirds (experiment 14).

We attempted to determine whether the variations in mucosal sterol synthesis might be related to hormonal variations accompanying the estrus cycle by studying mucosal scrapings taken from the distal third of the small intestine of rats in different phases of estrus. Although the lowest mean activity occurred during metestrus, the values for the several phases of the cycle overlapped so greatly that no conclusion could be reached.[1]

DISCUSSION

These studies demonstrate that isolated scrapings of mucosa from the small intestine of the guinea pig and rat retain the ability in vitro to incorporate ^{14}C-labeled acetate and mevalonate into sterols. The incorporation of each substrate into the C_{27}-sterols is more active in mucosa from the middle and distal thirds of the guinea pig intestine than in mucosa from the proximal third. Although rat mucosa is generally less active than that of the guinea pig, the localization of rat intestinal sterol synthesis to the ileum is striking. These regional differences in activity of incorporation could be due either to differences in one or more rate-limiting steps in the biosynthetic pathway, or to differences in cellular uptake of the labeled substrates. That the regional differences were observed for both substrates suggests, perhaps, that variation in activity of one or more steps in the pathway between mevalonate and the C_{27}-sterols is the more likely explanation.

Total C_{27}-sterol concentration varied only slightly along the intestine. In mucosa from all regions cholesterol was the major C_{27}-sterol, and its absolute concentration and its concentration relative to that of the other sterols increased distally. Despite the predominance of cholesterol, however, in no case was more than 5% of the acetate-^{14}C incorporated into C_{27}-sterols found in this sterol. At least 95% of the radioactivity was in lathosterol plus 7-dehydrocholesterol, with most of it in the former. The specific activities of the three sterols differed from one another significantly, and were consistent with the biosynthetic pathway: lathosterol to 7-dehydrocholesterol to cholesterol. Although determinations of pool sizes and specific activities at other time intervals in addition to the 2 hr period of the present study would be needed to establish this pathway with certainty, the conclusion is in agreement with those of others (19, 20). However, during incubations lasting from 1 to 4 hr. when incorporation of acetate into C_{27}-sterols was nearly linear, the percentage of radioactivity that appeared in cholesterol did not increase appreciably.

The accumulation of radioactivity in lathosterol and 7-dehydrocholesterol may reflect an artifact of the experimental conditions in vitro, although Schwenk et al. (4) found that acetate given to guinea pigs in vivo appeared primarily in intestinal sterols other than cholesterol. Moreover, in the present studies, in liver slices, about two-thirds of the radioactivity incorporated into C_{27}-sterols appeared in cholesterol. Posssibly the intestinal cholesterol pool is poorly labeled because isotope is trapped in the pools of lathosterol and 7-dehydrocholesterol. The present data do not permit a kinetic analysis of the pool turnovers, but they do suggest that this is not the only explanation. If it were, one would expect that for a given degree of incorporation of label into C_{27}-sterols, the appearance of label in the cholesterol pool would be inversely related to the sizes of the lathosterol and 7-dehydrocholesterol pools. However, in the studies of proximal and distal mucosa, the combined pools of the latter two sterols fell significantly along the intestine, but the percentage of radioactivity appearing in cholesterol did not increase but rather showed no change or decreased despite a higher over-all incorporation of isotope into C_{27}-sterols distally.

The origin of lathosterol and 7-dehydrocholesterol in intestinal mucosa appears to be synthesis in that tissue. The diet of guinea pigs is vegetable and contains essentially none of these sterols, and the mucosal concentration of 7-dehydrocholesterol is not altered by fasting (14). Since the liver appears to accumulate very little of these compounds, and since the bile of the rat, an animal which also has significant amounts of these sterols in its

[1] Ockner, R. K., and L. Laster, unpublished experiments.

intestinal mucosa, has been shown not to contain these sterols (21), it would appear that biliary sterols do not contribute to mucosal pools of lathosterol and 7-dehydrocholesterol. In contrast, cholesterol, which may be present in small amounts in the diet, is also a major constituent of bile and is actively absorbed. Thus it is clear that a significant portion of the mucosal cholesterol pool can be derived from dietary and biliary sterol. Because of this, and because of the evidence in the present studies suggesting that not all of the 7-dehydrocholesterol synthesized by intestinal mucosa is necessarily converted to cholesterol, it is possible that 7-dehydrocholesterol has one or more unknown additional fates. Wells, Coleman, and Baumann (21) showed that lathosterol and 7-dehydrocholesterol are present in feces of rat and guinea pig, and concluded that these fecal sterols were of intestinal origin. Glover, Glover, and Morton (14) speculated that 7-dehydrocholesterol functions as a precursor of vitamin D_3, and other metabolic fates are also possible.

Intestinal wall taken from proximal, middle, and distal thirds of guinea pig small intestine, and scraped free of mucosa, also incorporated acetate-^{14}C into C_{27}-sterols. The activities were compared with those of the corresponding mucosal scrapings and in two experiments no consistent relationship was observed between mucosa and intestinal wall from the same intestinal region, or among intestinal wall samples from different regions of the intestine.[1] The differences in sterol metabolism between mucosa and intestinal wall are further emphasized by the finding that the 7-dehydrocholesterol content of muscularis is less than 25% of that of the mucosa (14). These differences between intestinal mucosa and intestinal wall, and the differences observed for hepatic sterol metabolism emphasize the need for caution in interpreting studies of intestinal sterols in experiments in which intact animals or whole gut wall were utilized. The mucosal sterols appear to be both qualitatively and quantitatively distinct, and the possibly unique functions they serve may be obscured by failure adequately to take into account the contribution of sterols from liver or intestinal muscularis.

Since the completion of the present studies, Dietschy and Siperstein (8) presented evidence that mucosa scraped from rat ileum exceeded that from other regions of small intestine in its capacity to incorporate acetate-^{14}C into digitonin-precipitable sterols, and that this incorporation was greater in mucosa from animals subjected to prior biliary diversion. Preliminary studies in this laboratory (22) have shown an inhibitory effect in vitro by physiological concentrations of taurocholate and glycocholate on the incorporation of both acetate and mevalonate into C_{27}-sterols by guinea pig intestinal mucosa. These findings are thus mutually confirmatory of the importance of distal mucosa in intestinal sterol synthesis, and suggest that the process may be controlled by bile salts, which have been shown to be most actively absorbed in that region (23).

The relationship of intestinal sterol synthesis to intestinal function in general and to the sterol metabolism of the organism remains unclear. Lindsey and Wilson (24) showed that a small portion of the circulating cholesterol in the rat may be derived from mucosal synthesis. Human subjects who received triparanol, an inhibitor of reduction of the Δ^{24}-double bond of sterols, developed an abnormality of intestinal morphology very similar to that seen in gluten-sensitive enteropathy (25). Whether this effect reflects direct toxicity or is secondary to the disturbance in sterol metabolism is not certain.

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