The biosynthesis, absorption, and origin of cholesterol and plant sterols in the Florida land crab

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Abstract In order to study the biosynthesis, composition, and origin of sterols in the Florida land crabs, *Cardisoma guanhumi* (Latreille), we fed 17 male crabs either a cholesterol-free or a high cholesterol diet for 2 to 7 weeks. The origin of sterols in these crabs, whether from biosynthesis or from the diet, was determined by three procedures: the incorporation of isotopic mevalonate into the cholesterol when the diet was cholesterol-free; the absorption of isotopic cholesterol and sitosterol from the diet; the cholesterol and plant sterol concentrations of hepatopancreas, plasma, and muscle under conditions of cholesterol-free and high cholesterol diets. In addition, the interconversion of cholesterol and sitosterol was investigated. Dietary sterols of plant and animal sources were readily absorbed and provided the major source of sterols for this species of crab. The biosynthesis of cholesterol from mevalonate in this crab was minimal. However, cholesterol was synthesized from dietary sitosterol by dealkylation. Cholesterol and the three plant sterols (24ξ-methyl cholesterol, stigmasterol, and sitosterol) were found in the hepatopancreas, plasma, and muscle of the crab. Plant sterols contributed from 9 to 37% of the total sterols in the hepatopancreas, plasma, and muscle of the crabs fed a cholesterol-free diet.—**Douglass, T. S., W. E. Connor, and D. S. Lin.** The biosynthesis, absorption, and origin of cholesterol and plant sterols in the Florida land crab. *J. Lipid Res.* 1981. **22:** 961–970.

Supplementary key words cholesterol-free diet · high cholesterol diet · crustacean · hepatopancreas · gas–liquid chromatography–mass spectrometry · isotopic mevalonate · isotopic cholesterol · isotopic sitosterol · interconversion of sterols

While the pathways of sterol biosynthesis for vertebrates, including man, have been extensively studied, sterol biosynthesis in invertebrates has been less completely investigated. Vertebrates synthesize cholesterol from precursor molecules such as acetate and mevalonate (1). Among the invertebrates, the current evidence suggests that insects and crustaceans cannot synthesize cholesterol and, therefore, presumably require a source of dietary sterol (2, 3). It has even been suggested that the inability to synthesize cholesterol (or other sterols) from acetate might be a metabolic characteristic of the whole class of crustacea (3). Growing evidence indicates that the lack of synthetic pathway occurs generally among the Arthropoda (2, 4). However, all experiments to date investigating the possibility of sterol biosynthesis in crustaceans have utilized animals either fasted or fed a diet containing cholesterol. Specifically, no experiments have been performed using animals fed very low cholesterol diets.

Since both the fasting state (5–7) and dietary cholesterol (8, 9) inhibit cholesterol synthesis in vertebrates, the same considerations might also apply to crustaceans and, thus, could explain the past failures to demonstrate cholesterol biosynthesis. The design of the experiment now to be reported was to study the biosynthesis of cholesterol in Florida land crabs, *Cardisoma guanhumi* (Latreille), fed a cholesterol-free diet to obviate the problems from fasting and from the ingestion of dietary cholesterol. In addition, the absorption of dietary cholesterol and plant sterols and their interconversion were also investigated (2).

MATERIALS AND METHODS

Experimental animals

Seventeen male land crabs, identified as *Cardisoma guanhumi*, were obtained from Southern Florida. (Tropical Atlantic Marine Specimens, P.O.B. 62, Big Pine Key, FL). After arrival, the crabs were placed in fiberglass tanks measuring 4 ft × 3 ft × 2.5 ft (3–4 crabs per tank). The tanks were dry except for two 10-in diameter dishes of sea water per tank provided for the crabs to moisten their gills and for drinking. The crabs were numbered using an indelible marker on their dorsal carapaces. The weight of the crabs averaged 203 g (range 150–250 g). No crab was in the molting cycle. A photo period of 14 hr continuous

Abbreviation: TLC, thin-layer chromatography.

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light was established. The temperature was maintained near 28°C.

Diets

Both cholesterol-free and cholesterol-containing diets were used in these experiments. These diets were prepared using a specially formulated semi-synthetic diet (General Biochemical Inc., Chagrin Falls, OH), made more cohesive by the addition of 100 g of flour and 40 g of water to 900 g of finely powdered chow. The mixture was formed into one-half inch cubes and baked at 100°C for 45 min. The resulting chow could be easily held by the pincers of the crabs while being eaten. The composition of the semi-synthetic diet is depicted in **Table 1.** To determine the dietary sterol composition, four randomly-picked samples of the diet were ground to powder separately. The lipids of each sample were extracted with chloroform–methanol (10). The sterol content was analyzed by saponification, digitonin precipitation, and gas–liquid chromatography, the same procedures described in a subsequent "analytical procedure" section for crab blood, muscle, and hepatopancreas.

Isotopes

[4-^{14}C]Cholesterol and [2-^{14}C]mevalonate dibenzoylethylene diamine (DBED) salt (98% purity) were purchased from New England Nuclear Corp., Boston, MA. The [4-^{14}C]sitosterol (98% purity) was obtained from Amersham/Searle Corp., Arlington Heights, IL. The purity of the isotopic sterols was further verified by TLC before use (11).

TABLE 1. Composition of the semisynthetic diets

	High Cholesterol Diet	Cholesterol-free Diet
Ingredients (g/100 g)		
Soya assay protein	7.48	20.23
Egg yolk powder	36.60	0
Corn starch	14.00	50.61
Cane sugar	33.94	0
Corn oil	0	21.58
Salt mix (Phillips-Hart)	4.00	4.00
Vitamin supplement (GBI)	1.00	1.00
Non-nutritive fiber	2.58	2.58
Crystalline cholesterol	0.40	0
Sterols (mg/100 g)		
Sitosterol	0	142
24ξ-methyl cholesterol	0	44
Stigmasterol	0	14
Cholesterol	1200	0
Total	1200	200
Calories (cal/100 g)	462	469
% from fat	40.6	41.3
% from carbohydrate	44.0	43.2
% from protein	15.4	15.5

Experimental procedures

Cholesterol biosynthesis: Four crabs (#1–4) were given a cholesterol-free diet for 2–4 weeks. An aqueous solution of [2-^{14}C]mevalonate was prepared from the benzoylethylenediamine salt of mevalonate by adding sodium bicarbonate to free the benzoylethylenediamine, and then ethyl ether to remove the amine. Approximately 10 μCi of [2-^{14}C]-mevalonate in 1.5 ml of solution was injected into the hemoceoles of each of the four crabs over a period of 2 min. These crabs were not fasted before isotopic injection and, after injection, they continued to receive the cholesterol-free chow. Crab blood was obtained through the hemoceole with a syringe and needle. One to 9 days after isotopic injection, the crabs were killed by freezing. The carapace was removed. The hepatopancreas and muscle were dissected out, minced finely, placed in weighed beakers, and dried under vacuum at 70–80°C to a constant weight. The tissue lipids were extracted with chloroform–methanol (10). The sterols and their radioactivities in the lipid extract and plasma were analyzed by the methods that will be described in the subsequent "analytical procedure" section.

Cholesterol absorption: Two groups of crabs of five each consumed either a cholesterol-free (crabs #5–9) or a high cholesterol (crabs #13–17) diet for 2 weeks before feeding [4-^{14}C]cholesterol. The labeled cholesterol was dissolved in a small amount of alcohol and mixed into the chow cube during the chow preparation, as described previously. The isotopic concentration of the chow was 0.05 μCi/g of chow. These crabs were killed after consuming the isotopic chow daily for 4–33 days.

Sitosterol absorption: After 1 week of a cholesterol-free diet, [4-^{14}C]sitosterol was added in the diet of another group of crabs (crabs #10–12). The isotopic concentration of the chow was approximately 0.1 μCi/g of chow. These crabs were killed after 41 days of feeding.

Analytical procedures

To determine the sterol content and its radioactivity in crab plasma and tissues, plasma and lipid extracts of the hepatopancreas and muscle were saponified with alcoholic KOH. The nonsaponifiable material was extracted with hexane. Sterols were then precipitated by digitonin (12). The free sterols were recovered from digitonide by dissolving the precipitate in pyridine and extracting the free sterols with

ethyl ether (13). Aliquots of this solution were dried under nitrogen. Sterols were converted to trimethylsilyl ether derivatives and subjected to gas–liquid chromatography with cholestane as internal standard (14). The analyses were performed on a Hewlett-Packard high efficiency chromatograph, Model 7610A equipped with a hydrogen flame ionization detector. The column was a 4-ft glass U-tube, 4 mm I.D., packed with WHP (80/100 mesh) with a 3.8% film of SE-30 (methylsiloxane polymer). Temperatures of columns, flame detector, and injection port were 230, 250, and 280°C, respectively. Helium was used as the carrier gas at a flow rate of 75 ml/min. A Hewlett-Packard model 3370B integrator was used to obtain the retention time and peak area of each for identification and quantification. The structure of the sterols in the crabs was further confirmed by combined gas–liquid chromatography and mass spectrometry[3] (15). Another aliquot of the sterol solution was taken to measure its radioactivity in a Packard Tricarb liquid scintillation spectrometer equipped with absolute activity analyzer (Packard Instrument Co., Downers Grove, IL).

For the purification of free and esterified sterols, the lipid extract of hepatopancreas was subjected to thin-layer chromatography. The lipids were chromatographed on a pre-coated silica gel G plate, 20 × 20 cm, (Quantum Industries, Fairfield, NJ). The solvent system was hexane–chloroform–ethyl ether–acetic acid 80:10:10:1. The bands of free and esterified sterol were scraped off the plate and eluted with ethyl ether. The eluents of the thin-layer chromatography plates were hydrolyzed to the free form by saponification, converted to the trimethylsilyl ether derivatives of the sterol, and subjected to the gas–liquid chromatography system described earlier. Isotopic free and esterified cholesterol were used to monitor any losses. The recovery varied between 85–90%.

To determine the possible conversion between the cholesterol and sitosterol molecules in the crab, the lipid extracts of the hepatopancreas of crabs fed either [4-^{14}C]cholesterol for 33 days or [4-^{14}C]sitosterol for 41 days were subjected to a reversed phased TLC system that separated cholesterol and sitosterol (16). The mass and radioactivity in the respective bands were determined by the methods described previously. A standard mixture containing the same proportion of cholesterol, sitosterol, and either isotopic cholesterol or sitosterol as the lipid extract of the hepatopancreas in question was also analyzed simultaneously as control. All analyses were performed in duplicate.

[3] Combined gas–liquid chromatography and mass spectrometry were performed by Dr. M. K. G Rao of the Burnside Laboratories, University of Illinois, Champaign, IL 61802.

RESULTS

Sterols of the hepatopancreas, blood plasma, and leg muscle: dietary effects

The cholesterol and plant sterol contents of the hepatopancreas of the 12 crabs consuming a cholesterol-free, plant sterol-containing diet for 12 to 48 days were 1.10 ± 0.55 mg/g and 0.64 ± 0.34 mg/g dried tissue, respectively (**Table 2**). The plant sterols included 78% sitosterol and 22% 24ξ-methyl cholesterol, a pattern similar to that found in the diet. Stigmasterol was also found, but in smaller amounts. It contributed 1% or less of the total plant sterols of the hepatopancreas and yet was 7% of the dietary sterols.

On the other hand, in the five crabs fed a diet containing cholesterol but no plant sterols, the cholesterol and plant sterol contents of the hepatopancreas were different. The cholesterol was 4.79 ± 2.07 mg/g of dried tissue, significantly higher than the cholesterol-free diet groups ($P < 0.001$). The plant sterol content was somewhat lower (0.43 mg/g of tissue) but not statistically different from that of low cholesterol diet periods (0.64 mg/g of tissue). Sitosterol contributed 62% of the total plant sterols, and 24ξ-methyl cholesterol 38%. Muscle sterols, structural in nature, did not change in the crabs fed diets low or high in cholesterol content (**Table 3**). About half of the sterols in the hepatopancreas were esterified. Both cholesterol and sitosterol had about the same percent of esterification (50% and 46%, respectively).

Blood samples were collected from six crabs receiving a low cholesterol diet and three given a high cholesterol diet (**Table 4**). The blood cholesterol level of these crabs was significantly increased by high cholesterol diets (14.20 ± 3.07 mg/dl in high cholesterol diet period versus 6.96 ± 3.09 in low cholesterol diet periods, $P < 0.025$). The blood plant sterol concentration was 1.61 ± 0.76 mg/dl for the low cholesterol diet period and 0.68 ± 0.19 ($P < 0.1$) for the high cholesterol diet. To be noted is the fact that the high cholesterol diet contained no plant sterols in contrast to the considerable dietary plant sterol intake during the low cholesterol diet (Table 1). Total plant sterol concentration in the blood during the low cholesterol diet period was somewhat higher than that during the high cholesterol diet period (1.61 ± 0.76 versus 0.68 ± 0.19, $P < 0.1$). The plasma cholesterol

TABLE 2. The sterol content of the hepatopancreas of Florida land crabs consuming cholesterol-free and high cholesterol diets

Crab #	Diet	Days of Diet	Sterol Content					
				Plant Sterols				
			Cholesterol	24ξ-Methyl Cholesterol	Stigmasterol	Sitosterol	Total	Total Sterols
			mg/g dried tissue					
1	Cholesterol-free	12	2.51	0.18		1.15	1.33	3.84
2	Cholesterol-free	34	1.38	0.10		0.60	0.71	2.08
3	Cholesterol-free	18	0.94	0.09		0.53	0.62	1.55
4	Cholesterol-free	41	1.08	0.12		0.48	0.60	1.68
5	Cholesterol-free	48	1.46	0.41		0.78	1.19	2.65
6	Cholesterol-free	48	1.33	0.15		0.50	0.65	1.98
7	Cholesterol-free	48	1.24	0.21		0.55	0.77	2.01
8	Cholesterol-free	18	0.71	0.09	0.02	0.50	0.60	1.32
9	Cholesterol-free	19	0.80	0.04		0.30	0.34	1.14
10	Cholesterol-free	48	0.67	0.08		0.19	0.27	0.94
11	Cholesterol-free	48	0.49	0.09		0.19	0.28	0.78
12	Cholesterol-free	48	0.58	0.08		0.21	0.29	0.88
Mean ± S.D.			1.10 ± 0.55	0.14 ± 0.10		0.50 ± 0.28	0.64 ± 0.34	1.74 ± 0.87
13	High cholesterol	37	3.83	0.14		0.34	0.47	4.30
14	High cholesterol	41	8.33	0.22		0.30	0.52	8.85
15	High cholesterol	43	4.83	0.17		0.22	0.39	5.22
16	High cholesterol	46	3.29	0.18		0.30	0.47	3.77
17	High cholesterol	47	3.65	0.10		0.16	0.26	3.92
Mean ± S.D.			4.79 ± 2.06	0.16 ± 0.05		0.26 ± 0.07	0.43 ± 0.10	5.21 ± 2.11
P Value (Cholesterol-free vs. high cholesterol)			<0.001	N.S.		<0.1	N.S.	<0.001

and sitosterol maintained the same ratio, one to the other, regardless of their total values.

Incorporation of [2-^{14}C]mevalonate into cholesterol

The hepatopancreas of four crabs were analyzed 1–9 days after they had received approximately 10 μCi of [2-^{14}C]mevalonate. The radioactivity in the nonsaponifiable material ranged from 4,506 to 25,348 dpm/g dried tissue. In comparison, the radioactivity in the digitonin-precipitable sterol fraction was low, 98 to 690 dpm/g dried tissue, or only 3% of the total counts in the nonsaponifiable fraction (**Table 5**). The mass of the cholesterol in these two fractions (nonsaponifiable material and digitonin-precipitable sterols), however, was the same. Thus, the synthesis of cholesterol from mevalonate was minimal to absent in crabs fed a cholesterol-free diet.

Absorption of [4-^{14}C]cholesterol from the diet

Five crabs were fed cholesterol-free diets containing 0.05 μCi [4-^{14}C]cholesterol per g chow for 4–33 days. The significant radioactivity (1,186–15,975 dpm/g of dried tissue) found in the sterol fraction of the hepatopancreas of these crabs (**Table 6**) indicated considerable cholesterol absorption. Another five crabs were given dietary cholesterol (1200 mg/100 g of chow) with [4-^{14}C]cholesterol for 23–33 days. The radioactivity in the hepatopancreas of these crabs ranged 23,489–37,126 dpm/g dried tissue, much higher than in the previous group fed cholesterol-free diets. The mean cholesterol specific activity in the hepatopancreas of three crabs fed isotopic cholesterol with cholesterol-free diets for 27–33 days was 27,572. For the five crabs fed the same amount of isotope but with a high cholesterol diet for the same period of time, the specific activity was 7,354. Since the specific activity of the dietary cholesterol was 9,250, the ratio of the cholesterol specific activities of the hepatopancreas and the diet indicated that about 79.4% of its cholesterol was derived from the diet. Since there was virtually no cholesterol synthesis in these crabs, the other 21% of the cholesterol must have been present in the hepatopancreas before our experiments were begun. These data permit an estimation of cholesterol turnover in the hepatopancreas. This was calculated to be 37 days.

Absorption of [4-^{14}C]sitosterol from the diet

[4-^{14}C]Sitosterol was incorporated into the crab diet in the amount of 0.1 μCi/g. This chow contained no cholesterol and 142 mg sitosterol per 100 g. Three

TABLE 3. The sterol content of the leg muscle of the Florida land crab consuming cholesterol-free and high cholesterol diets

Crab #	Diet	Days of Diet	Sterol Content					
				Plant Sterols				
			Cholesterol	24ξ-Methyl Cholesterol	Stigmasterol	Sitosterol	Total	Total Sterols
			mg/g dried tissue					
1	Cholesterol-free	12	2.22	0.03		0.13	0.16	2.39
2	Cholesterol-free	34	1.42	0.04		0.12	0.16	1.58
3	Cholesterol-free	18	0.71	0.01		0.04	0.06	0.76
4	Cholesterol-free	41	1.69	0.05		0.12	0.17	0.81
5	Cholesterol-free	18	1.06	0.04		0.08	0.11	1.17
6	Cholesterol-free	19	1.71	0.03		0.12	0.15	1.86
7	Cholesterol-free	48	1.43	0.05		0.09	0.14	1.57
8	Cholesterol-free	48	1.46	0.08		0.11	0.18	1.65
9	Cholesterol-free	48	1.74	0.07		0.11	0.18	1.85
Mean ± SD			1.50 ± 0.43	0.04 ± 0.02		0.10 ± 0.03	0.15 ± 0.04	1.63 ± 0.46
13	High cholesterol	37	1.87	0.06	0.02	0.09	0.17	2.04
14	High cholesterol	41	1.43	0.02		0.06	0.08	1.51
15	High cholesterol	43	1.85	0.02		0.06	0.08	1.92
16	High cholesterol	46	1.84	0.03		0.09	0.12	1.96
17	High cholesterol	47	1.60	0.01		0.06	0.07	1.67
Mean ± SD			1.72 ± 0.19	0.03 ± 0.02		0.07 ± 0.02	0.10 ± 0.04	1.81 ± 0.22
P value (Cholesterol-free vs. high cholesterol)			N.S.	N.S.		$P < 0.05$	N.S.	N.S.

crabs were fed this labeled diet for 41 days. At that time significant radioactivity (9,768–33,667 dpm/g dried tissue) was detected in the sterol fraction of the hepatopancreas (**Table 7**). The mean sitosterol specific activity was 28,035 dpm/mg sterol. The specific activity of the dietary sitosterol was 156,338 dpm/mg. The ratio of the specific activity of hepatopancreas and diet indicated that after 41 days feeding of the cholesterol-free, sitosterol-containing diet, only 17.9% of the sitosterol of the hepatopancreas was derived from dietary beta-sitosterol. Based on the same rationale described in the previous paragraph for cholesterol, the turnover time for sitosterol in hepatopancreas is 229 days.

Conversion between cholesterol and sitosterol

To test the possibility of interconversion of cholesterol and sitosterol, we analyzed the mass and radio-

TABLE 4. The sterol content of the blood plasma of the Florida land crab consuming cholesterol-free and high cholesterol diets

Crab #	Diet	Days of Diet	Sterol Content					
				Plant Sterols				
			Cholesterol	24ξ-Methyl Cholesterol	Stigmasterol	Sitosterol	Total	Total Sterols
			mg/dl					
1	Cholesterol-free	12	10.34	0.50		1.37	1.87	12.21
2	Cholesterol-free	34	10.51	0.38		2.17	2.55	13.06
3	Cholesterol-free	18	3.98	0.13		0.92	1.05	5.03
4	Cholesterol-free	41	5.91	0.17		1.06	1.23	7.14
5	Cholesterol-free	18	7.71	0.28		2.06	2.34	10.05
7	Cholesterol-free	41	3.33	0.10		0.54	0.64	3.97
Mean ± SD			6.96 ± 3.09	0.26 ± 0.16		1.35 ± 0.65	1.61 ± 0.76	8.58 ± 3.78
14	High cholesterol	41	16.02	0.07		0.59	0.66	16.68
16	High cholesterol	46	10.65	0.08		0.80	0.88	11.54
17	High cholesterol	47	15.93	0.05		0.46	0.51	16.44
Mean ± SD			14.20 ± 3.07	0.07 ± 0.02		0.62 ± 0.17	0.68 ± 0.19	14.90 ± 2.91
P value (Cholesterol-free vs. high cholesterol)			<0.025	N.S.		N.S.	N.S.	<0.05

TABLE 5. The incorporation of [2-^{14}C]mevalonate into the nonsaponifiable material and digitonin-precipitable sterols of hepatopancreas of Florida land crabs

Crab #	Days after Isotopic Administration	Radioactivities[a] in Nonsaponifiable Extract	Radioactivities[a] in Digitonin-precipitable Sterols
		(dpm/g dried tissue)	
1	1	25,348	690
2	3	4,506	98
3	7	7,802	410
4	9	15,793	509

[a] The mass of cholesterol was the same in the nonsaponifiable material and digitonin-precipitable sterol.

activity of cholesterol and sitosterol in the hepatopancreas of two crabs (crabs #9 and 17) fed [4-^{14}C]-cholesterol with cholesterol-free and high cholesterol diets for 33 days, and three crabs (crabs #10–12) fed [4-^{14}C]sitosterol with a cholesterol-free diet for 41 days. The cholesterol and sitosterol were separated by a reverse TLC system. In order to control crossover contamination during their separation, a standard mixture containing similar concentrations of cholesterol, sitosterol, and either isotopic cholesterol or sitosterol, dependent upon which isotope was present in the crab samples, was always analyzed simultaneously with the sample.

In the hepatopancreas of the crabs fed [4-^{14}C]-cholesterol for 33 days, the radioactivity was almost completely confined to the cholesterol band. The radioactivity in the sitosterol band was 1.2% of the total radioactivity for crab #9, 2.1% for crab #17, and 5.2% for standard mixture. The small amount of radioactivity found in the sitosterol band was likely derived from crossover contamination of the isotopic cholesterol during the separation process rather than the conversion of isotopic cholesterol to sitosterol.

On the other hand, the radioactivity in the cholesterol band of hepatopancreas of three crabs fed [4-^{14}C]sitosterol for 41 days amounted to 10–25% (mean 17%) of the total radioactivity (**Table 8**). For the standard mixture run simultaneously, the radioactivity in the cholesterol band was only 3% of the total radioactivity (counted for crossover contamination by [4-^{14}C]sitosterol during the separation). Therefore, the high amount of radioactivity found in the cholesterol band of the hepatopancreas of crabs fed [4-^{14}C]sitosterol could not be accounted for by contamination from isotopic sitosterol during the separation process. In addition, the sterol content of the cholesterol bands was analyzed by gas–liquid chromatography and no sitosterol was detected. From this evidence, we concluded that cholesterol was synthesized, in part, from absorbed dietary sitosterol in this crustacean.

We calculated from the data presented in Table 8 that, at a minimum, 8.7% of the cholesterol in the hepatopancreas was synthesized from sitosterol over a 41-day period. This figure was derived from the amount of radioactive cholesterol formed from the administered radioactive sitosterol. The mean specific activities in the hepatopancreas were 1732 dpm/mg and 19,879 dpm/mg for cholesterol and sitosterol, respectively. Since the cholesterol content of the hepatopancreas was 4.51 mg, the minimum conversion rate was estimated roughly to be 9.6 μg/day. We assume that the remainder of the cholesterol in the hepatopancreas was derived from earlier ingestion of dietary cholesterol and of dietary sitosterol converted to cholesterol before our experiments were initiated.

TABLE 6. The absorption of [4-^{14}C]cholesterol from the diet by Florida land crabs[a]

Crab #	Diet	Days after the Isotopic Diet Feeding	[4-^{14}C]Cholesterol Content in Nonsaponifiable Material	[4-^{14}C]Cholesterol Content in Digitonin-precipitable Sterols	Cholesterol Specific Activity
			dpm/g of hepatopancreas		*dpm/mg cholesterol*
5	Cholesterol-free	4	1,298	1,186	1,666
6	Cholesterol-free	5	2,643	2,535	3,165
7	Cholesterol-free	27	15,984	15,725	23,400
8	Cholesterol-free	27	16,042	15,766	31,915
9	Cholesterol-free	33	16,289	15,975	27,401
13	High cholesterol	23	25,011	23,489	6,129
14	High cholesterol	27	66,123	64,713	7,769
15	High cholesterol	29	38,684	37,126	7,685
16	High cholesterol	32	24,001	22,848	6,941
17	High cholesterol	33	31,825	30,125	8,248

[a] These crabs were given a diet containing [4-^{14}C]cholesterol (0.05 μCi/g of chow).

TABLE 7. The absorption of [4-^{14}C]sitosterol from the diet by the Florida land crab[a]

Crab #	Radioactivities in Nonsaponifiable Material	Radioactivities in Digitonin-precipitable Sterol	Specific Activity
	dpm/g dry hepatopancreas		*dpm/mg sitosterol*
10	34,781	33,667	43,274
11	11,527	9,798	19,596
12	11,874	11,765	21,236

[a] These crabs were fed a diet containing [1-^{14}C]sitosterol (approximately 0.1 μCi/g of chow) for 41 days.

DISCUSSION

In previous experiments, cholesterol biosynthesis did not occur after the administration of either [1-^{14}C]acetate and/or [2-^{14}C]mevalonate in the crabs, *Cancer pagurus* (17) or *Portunus trituberculatus* (18), the crayfish, *Astacus astacus* (19–22), the lobsters, *Homarus gammarus* (23, 24) or *Panulirus japonica* (17), or the prawn, *Penaeus japonicus* (18). However, the animals in these experiments were either fasted or fed a diet containing a significant amount of cholesterol. Both of these conditions would inhibit cholesterol synthesis (5–9).

Wieland et al. (7) noticed a marked decrease in the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase in fasting rats which is thought to explain reduced synthesis of cholesterol in the fasting state. This enzyme is involved in the feedback control mechanism whereby cholesterol inhibits cholesterol synthesis by the liver (25). Morris and Chaikoff (9) found that endogenous cholesterol production was inhibited by as little as 0.05% cholesterol in the diet and that 2% dietary cholesterol inhibited endogenous cholesterol synthesis by up to 90% in the rat.

To avoid the possible effects of these dietary conditions upon the outcome of cholesterol biosynthesis in the present study, we fed the Florida land crab a cholesterol-free diet continuously and then studied the incorporation of [^{14}C]mevalonate into the cholesterol molecule. Our data showed that even under these most favorable conditions, cholesterol biosynthesis in these crabs was minimal. Plant sterols also were not synthesized. This result is consistent with the view that crustaceans, as a whole, are incapable of cholesterol synthesis from mevalonate (3). Zandee (24) has suggested that this inability of crabs to synthesize cholesterol is possibly correlated with the inability of crabs to degrade cholesterol to bile acids. The precise metabolic block in the sterol synthetic pathway in these crustaceans is still unknown.

Because of this inability of crustaceans to synthesize cholesterol, it was assumed that there must be a dietary requirement for sterols in these shellfish. The predominant sterol, cholesterol, then would be of dietary origin (3, 18). Our data showed that both cholesterol and sitosterol were readily absorbed from the diet by these crabs. Not only was the absorption of sterols demonstrated by the appearance of isotopic sterols in the tissues, but this feature was also

TABLE 8. The separation of cholesterol and β-sitosterol in the hepatopancreas of crabs fed [4-^{14}C]sitosterol and a cholesterol-free diet for 41 days

	Standard Mixture[a]	Crab #10[a]	Crab #11[a]	Crab #12[a]
Sterol radioactivities (dpm)				
In the cholesterol band	22	237	84	59
In the sitosterol band	636	726	452	514
Crossover	3.4%	24.6%	15.7%	10.3%
Sterol mass (μg)				
In the cholesterol band				
Cholesterol	83.0	74.0	66.0	82.0
24ξ-methyl cholesterol	0	3.0	3.5	1.2
Sitosterol	0	0	0	0
In the sitosterol band				
Cholesterol	2.6	2.8	2.9	1.6
24ξ-methyl cholesterol	0	2.0	1.4	1.7
Sitosterol	45.0	26.0	31.0	30.0
Specific activities (dpm/mg)				
Cholesterol		3,202	1,273	720
Mean value			1,732	
Sitosterol		27,923	14,581	17,133
Mean value			19,879	

[a] Mean of duplicate analyses.

demonstrated by mass changes. When the diet was changed from a cholesterol-free to a high cholesterol diet, a significant increase in the cholesterol concentration in both the blood and the hepatopancreas was observed. When the crabs were given a 1200 mg cholesterol per 100 g diet for 23–33 days, 79.4% of cholesterol in the hepatopancreas was derived from the diet. Teshima et al. (29, 30) also found that dietary cholesterol and several C-24 alkylated sterols were very efficiently absorbed by *P. japonicus* and by the crabs, *Sesarma dehaani* and *Helice tridens*. As it has been shown that sterols are needed for the growth of crustaceans (26–28), the available dietary sterols (both cholesterol and plant sterols) must play an important role in supplying the sterol needs of the Florida land crab.

The similar ratios of 24ξ-methyl cholesterol, stigmasterol, and sitosterol in the diet and in the hepatopancreas of crabs fed this diet further suggested a dietary origin of these plant sterols. In the rat and human, the ability to absorb sitosterol is limited and estimated to be less than one-tenth of that for cholesterol (31–33). When the crabs were given a diet containing 142 mg sitosterol plant sterols per 100 g of chow for a longer period of time (41 days), only 17.9% of the sitosterol in the hepatopancreas (total 0.20 mg/g dried tissue) was derived from the diet. From this comparison, the absorption of plant sterols in the crab appeared similar to that in the rat and human, i.e., lower than for cholesterol.

While cholesterol was the predominant sterol in the hepatopancreas of the Florida land crab, the presence of large amounts of plant sterols (37% of total hepatopancreas sterols) in this study was a new finding. Until this study, the crab, *Cancer pegurus*, has been the only crustacean reliably shown to contain significant amounts of sterols other than C27-sterols (34). The presence of sitosterol in a crustacean has been previously reported only in the Alaskan king crab, *Paralithodes camtschatica*, and only then in trace amounts, 0.8% of the total sterol (35). Cholesterol was the only significant measurable sterol in certain other crabs: *Portunus trituberculatus* (18), *Hemigrapsus nudus*, and numerous other species (36). Bergman (36) and Austin (34) have both suggested that cholesterol may be the predominant sterol in all crabs.

However, in the human species sitosterol has been found in high concentrations in the blood and tissues. A genetic disease has been described in a number of patients with clinical consequences of xanthoma and atherosclerosis (37, 38). These patients readily absorb plant sterols as does the Florida land crab. Parallel changes of cholesterol and plant sterol in the blood of these patients were also observed. The precise mechanism behind this phenomenon is not yet known and requires further study.

In a recent review article, Goad (3) reported that in every species of crustacean examined, cholesterol is the major constituent, usually in excess of 90% of the total sterols. In those species with a cholesterol content of less than 90% of total sterols, the balance is usually made up by the related C_{27}-sterol, desmosterol. Kritchevsky et al. (39) found that the crab, *Cancer pagurus*, contained significant amounts of other sterols besides cholesterol (cholesterol 57%, brassicasterol 37% 22-dehydrocholesterol 4% and 24-methylene cholesterol 2%). The muscle of the Alaskan king crab, *Paralithodes camtschatica*, contained two major sterols, cholesterol 62.3% and desmosterol 37.1%, and trace amounts of other sterols that included 0.8% sitosterol (35). In the same paper, Idler and Wiseman reported that the muscle sterols from the North Atlantic queen crab, *Chionoeoetes opiolo*, contained 93.6% cholesterol and 6.1% desmosterol.

The Florida land crab is an omnivorous, predominantly terrestrial creature known to feed on grasses and leaves in addition to animal products. In view of the fact that plant sterols are readily absorbed by these crabs, and that this crab is incapable of synthesizing sterols, the large amount of plant sterols present in the tissue is most likely of dietary origin.

The conversion between cholesterol and sitosterol in the Florida land crab was studied in these experiments. Our data indicated that there was dealkylation of sitosterol to cholesterol but no conversion of cholesterol to sitosterol. Dealkylation of 24-methyl and 24-ethylesterol to cholesterol has been shown in other crustaceans. The conversion of ergosterol, brassicasterol, and campesterol to cholesterol in *Artemia salina* (40–42), sitosterol to cholesterol in prawn, *Penaeus japonicus* (41), and ergosterol to cholesterol in crab, *Portunus trituberculatus* (43), has been reported by Teshima and Teshima and Kanazawa. While the dealkylation of 24-methyl and 24-ethyl sterols in insects is well established (2, 3) and several aspects of the mechanisms have now been elucidated (44, 45), the information about the dealkylation mechanisms operative in crustaceans is still very limited. In the tobacco hornworm, *Manduca sexta*, it has been demonstrated that desmosterol is an intermediate in the conversion of sitosterol to cholesterol (46–48). Teshima and Kanazawa found that a prawn (*Penaeus japonicus*), a shrimp (*Palaemen serratus*), and a crab (*Sesarma dohanni*) can reduce desmosterol to cholesterol (30, 49, 50). These findings suggested the probability that the same dealkylation route operative in insects may also operate in the crustaceans. However, in our experiments, no

desmosterol was detected in the crab tissues. The mechanism of dealkylation in the crustaceans remains for future studies.

Supported by research grants from the National Institute of Arthritis and Metabolic Disease (AM-21478), National Heart, Lung, and Blood Institute (training grant HL 07295), and the Clinical Research Center Program (RR 334) of the National Institutes of Health.

Manuscript received 17 December 1980 and in revised form 30 April 1981.

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