Effect of cholesterol feeding and fasting on sterol synthesis in seventeen tissues of the rat

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ABSTRACT Rates of sterol synthesis were measured in 17 tissues of the rat, and the responsiveness of these rates to cholesterol feeding and to fasting was determined. The liver and gastrointestinal tract together account for 90% of synthetic activity of the whole body. After the rats had been fed cholesterol or fasted, liver synthesis was markedly decreased, whereas synthetic rates in all other organs tested were essentially unaffected (this conclusion applies to synthesis of cholesterol and of five other digitonin-precipitable tissue sterols). Consequently, the highest rate of cholesterogenesis in the cholesterol-fed or fasted rat is found in the gastrointestinal tract.

KEY WORDS rat · cholesterol synthesis · liver · intestine · extrahepatic tissues · feedback control · intermediate sterols · fasting · cholesterol feeding

SINCE THE INITIAL demonstration by Bloch, Borek, and Rittenberg in 1946 that surviving rat liver is able to synthesize digitonin-precipitable sterols from labeled acetate (1), virtually every mammalian tissue so far tested has been shown to be capable of cholesterogenesis (2). However, primary interest in this field has remained centered upon hepatic sterol synthesis because of early experimental evidence which suggested that the liver was the major source of circulating cholesterol (3–6). Furthermore, several investigators showed that the rate of hepatic cholesterogenesis could be drastically reduced either by cholesterol feeding or by fasting, a finding which strongly implicated these two dietary manipulations as major factors in the physiologic control of over-all sterol balance in the intact animal (3, 7–9). Data presented in several recent publications from this laboratory have demonstrated that the primary site of suppression of hepatic sterol synthesis brought about by the cholesterol feedback system is at a single enzymatic step in the cholesterol biosynthetic pathway, i.e., the conversion of β-hydroxy-β-methyl glutarate to mevalonic acid by the enzyme β-hydroxy-β-methyl glutaryl reductase (10, 11). Depressed activity of this same enzyme has also been suggested as the cause of the reduced rate of sterol synthesis found in the livers of fasted animals (12, 13).

Thus, while fasting and the cholesterol content of the diet have both been shown to influence cholesterogenesis by the liver markedly, only a few studies have appeared in which control mechanisms have been evaluated in extrahepatic tissues (10, 14–16). Since the rate of cholesterogenesis in several of these tissues approaches the synthetic rate of the liver itself, and since in man a significant portion of the endogenously produced circulating cholesterol pool arises in tissues other than the liver (17), there is need for a more thorough understanding of the regulation of sterol synthesis in these organs.

The primary purpose of the present investigation was to evaluate systematically the responsiveness of the cholesterol synthetic pathway in every major extrahepatic tissue both to prolonged cholesterol feeding and to fasting. In addition during the course of these experiments data were obtained which allowed an evaluation of two other aspects of sterol synthesis: first, the pattern of ^{14}C-acetate incorporation into specific intermediate sterols was determined in each different organ; and second, the magnitude of the "whole organ" synthesis of digitonin-precipitable sterols as well as that specifically of cholesterol was calculated for each of these various tissues. These experiments therefore provide information upon which one can make a critical comparison of sterol synthesis in the different tissues of the rat with respect to (*a*) the rate of synthesis of digitonin-precipitable sterols per unit weight of tissue, (*b*) the magnitude

Abbreviations: TLC, thin-layer chromatography; GLC, gas–liquid chromatography.

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of total sterol synthesis in the whole organ, (*c*) the pattern of acetate-2-^{14}C incorporation into intermediate sterols, and (*d*) the effect, or lack of effect, of cholesterol feeding and of fasting upon the rate of cholesterogenesis.

METHODS AND MATERIALS

Animal Preparations

Only male Sprague-Dawley rats weighing 200–250 g were used in these experiments; however, preliminary studies demonstrated that there was no significant difference between the data obtained in male or female animals with respect to the rates or patterns of ^{14}C-acetate incorporation into the various sterols. Three experimental groups of animals were used: (*a*) control animals were maintained for 6 wk on a low cholesterol, ground rat-chow diet, to each 100 g of which 5 g of oleic acid was added; (*b*) cholesterol-fed animals were maintained for the same period of time on a ground rat-chow diet supplemented with 5% cholesterol dissolved in an equal weight of oleic acid; and (*c*) fasted animals were deprived of food but allowed water ad lib. for 48 hr before being killed.

Tissue Preparation and Incubation

The animals were killed by decapitation and the various organs were immediately excised, washed in cold saline, and placed in beakers of oxygenated Krebs bicarbonate buffer solution at 0°C. Tissue slices 1 mm thick of brain, lung, heart, esophagus, stomach, duodenum, jejunum, ileum, transverse colon, kidney, thigh muscle, and spleen were then prepared by means of a McIlwain tissue slicer. The testicular tubules were teased apart and incubated without further preparation. Skin was obtained from the low back of each rat after shaving off the hair; slices were prepared by hand. The adrenals were also sliced by hand into four or five pieces. We obtained intestinal smooth muscle by vigorously scraping the mucosal surface of an opened small bowel segment with the edge of a glass slide to remove all of the mucosal elements.

500 mg of each tissue, except the adrenals, was placed in incubation flasks containing 1 μc of acetate-2-^{14}C, 5 μmoles of sodium acetate, and 5 ml of Krebs bicarbonate buffer previously gassed with O_2–CO_2 95:5 (pH = 7.4). The two adrenal glands from each animal were combined, weighed, and placed in an incubation flask. The flasks were then gassed with the same O_2–CO_2 mixture, capped, and incubated in a metabolic shaker at 37°C for 2 hr.

Isolation of the ^{14}C-Labeled Sterols

The method used in our laboratory for the isolation of digitonin-precipitable sterols is discussed in detail elsewhere (10). Briefly, this procedure involves saponification of the contents of the incubation flasks followed by extraction of the total unsaponifiable lipids with petroleum ether and the subsequent precipitation of the 3β-hydroxy sterols with digitonin. After dehydrating the precipitates with acetone and washing them with diethyl ether, we dissolved the sterol digitonides in methanol and counted an aliquot of this solution in a scintillation fluid consisting of 0.015% *p*-bis[2-(5-phenyloxazolyl)]-benzene and 0.30% 2,5-diphenyloxazole in toluene (POPOP–PPO solution). All samples were counted in a Packard liquid scintillation counter, model 314E. Results are expressed as mμmoles of added acetate-2-^{14}C incorporated into digitonin-precipitable sterols per gram of tissue per 2 hr incubation period. For TLC and GLC the free sterols were regenerated from the digitonides by the method of Sperry (18). In this procedure the precipitate of sterol digitonides is dissolved in pyridine and the free sterols are extracted with diethyl ether. After being dried in vacuo over concentrated sulfuric acid (which removes the pyridine), the free sterols are redissolved in chloroform for chromatographic analysis as described below.

TLC

Three separate thin-layer chromatographic systems were employed for the separation of digitonin-precipitable sterols into specific sterol fractions.

(*a*) Benzene–ethyl acetate 5:1. Glass plates, 10 × 40 cm, were coated with a suspension of Silica Gel G (Brinkmann Instruments Inc., Westbury, N. Y.) in distilled water, 50 g:100 ml, and activated in an oven for 1 hr at 100°C. After standard and tissue sterols had been applied, the plates were developed for 4–6 hr in benzene–ethyl acetate 5:1 until the solvent front had run a full 35 cm. These long plates gave good separation of four major groups of sterols: cholesterol plus cholestanol, lanosterol, Δ^7-cholestenol, and Δ^7- plus Δ^8-methostenols.

(*b*) Benzene–ethyl acetate 20:1. Although TLC system (*a*) generally gave excellent separation of the digitonin-precipitable sterols, in the extracts from some tissues a very heavy cholesterol spot would occasionally tail slightly and be incompletely separated from the slower running Δ^7-cholestenol area. When that happened, we could always resolve these two areas completely by applying the sterols again to 10 × 40 cm plates coated with Silica Gel G and developing them in benzene–ethyl acetate 20:1. A paper pad was clamped to the top of each of these plates and the developing tanks were sealed so that the developing fluid would continuously ascend for 36 hr. Both of the TLC systems described represent variations of methods previously developed by Avigan, Goodman, and Steinberg (19). In both

systems >95% of the ^{14}C-sterols applied to the plates could be recovered.

(*c*) Benzene–hexane 15:85. This system was employed for the separation of cholestanol from cholesterol. 20 × 20 cm plates were coated with a 50% suspension of Silica Gel G in a 12% silver nitrate solution. After activation in the usual manner, standard and tissue sterols were applied and the plates were developed in benzene–hexane 15:85 for 36 hr. This method separated cholestanol from the slower-moving cholesterol (Reinke, R. T., and J. Wilson, personal communication).

After the completion of chromatography in any of these systems, the plates were dried, sprayed with a 0.005% Rhodamine G solution (National Aniline Division, Allied Chemical Corp., N. Y.), and examined under UV light. For assay of the ^{14}C content of each sterol, the individual spots were scraped from the plates and put directly into counting vials containing POPOP–PPO scintillation fluid. Quenching was corrected for by internal standardization. If the sterols were to be further analyzed by GLC rather than assayed for ^{14}C content, individual spots were scraped into centrifuge tubes and the sterols were extracted with chloroform and then taken to dryness under nitrogen.

GLC

GLC of standard and tissue sterols was carried out in a Research Specialties Instrument (Research Specialties Co., Richmond, Calif.): the 6 ft column was packed with Gas-Chrom P coated with 1% SE-30 (methylpolysiloxy gum, General Electric); the gas phase was argon at a flow of 80–100 ml/min; and the column temperature was 230°C.

RESULTS

Rates of Sterol Synthesis in Tissues of the Control Rat

Initial experiments were undertaken to determine the relative rates of sterol synthesis of each tissue of the normal control rat. Shown in column A of Table 1 are the mean rates found in four such animals. The tissues have been arranged in order of descending synthetic activity.

As is apparent from the data, one can roughly divide these tissues into three groups on the basis of their synthetic ability. Clearly, the tissues which most actively incorporate acetate-2-^{14}C into sterols are the liver and three portions of the gastrointestinal tract: the ileum, colon, and stomach. Surprisingly, the ileum, which has the second highest incorporation rate of any of the tissues tested, synthesizes sterols at nearly 65% of the rate found in the liver. By contrast, the remainder of the thoracic and abdominal viscera—including the esophagus, lungs, duodenum, jejunum, adrenals, spleen, and kidneys as well as the testes and skin—all have relatively low synthetic rates: 3.5–17.4 mμmoles/2 hr or only about 2–10% of the hepatic incorporation rate. Finally, there is a third group of tissues which, while they definitely synthesize some sterol, do so at the exceedingly low incorporation rate of less than 1.0 mμmole of acetate-2-^{14}C per g of tissue per 2 hr (less than 0.6% of the hepatic rate). This group includes all three types of muscle, i.e., striated, smooth, and cardiac, as well as the brain.

TABLE 1 RATES OF STEROL SYNTHESIS IN VARIOUS TISSUES OF CONTROL, CHOLESTEROL-FED, AND FASTED RATS

Values are mμmoles (mean for 4 rats ± 1 SD) of acetate-2-^{14}C converted to digitonin-precipitable sterols per gram of tissue during a 2 hr incubation. Tissues are listed in column A in descending order of synthetic activity.

Tissue	A. Control	B. Fed Cholesterol for 6 Wk	C. Fasted for 48 Hr
Liver	179.0 ± 97.5	1.2 ± 0.3	15.9 ± 3.3
Ileum	114.3 ± 55.1	89.5 ± 17.0	69.2 ± 9.7
Transverse colon	56.2 ± 31.6	60.0 ± 4.0	33.2 ± 6.2
Stomach	35.7 ± 10.5	42.1 ± 15.2	25.2 ± 8.0
Esophagus	17.4 ± 7.3	12.6 ± 8.0	13.7 ± 5.9
Jejunum	15.2 ± 10.0	8.0 ± 5.2	7.5 ± 2.0
Testis	8.7 ± 2.7	9.6 ± 4.8	11.1 ± 0.6
Lung	6.7 ± 0.2	4.5 ± 2.7	5.2 ± 1.5
Adrenal	5.0 ± 1.1	5.6 ± 2.7	8.4 ± 6.0
Skin	4.9 ± 1.8	5.4 ± 1.7	3.6 ± 0.5
Kidney	3.8 ± 3.1	2.2 ± 0.6	2.2 ± 1.3
Duodenum	3.6 ± 1.6	2.0 ± 1.8	2.1 ± 0.5
Spleen	3.5 ± 0.7	4.2 ± 1.2	2.0 ± 1.3
Smooth muscle	0.9 ± 0.3	0.5 ± 0.4	0.6 ± 0.2
Heart muscle	0.7 ± 0.2	0.5 ± 0.2	0.4 ± 0.2
Skeletal muscle	0.5 ± 0.2	0.7 ± 0.3	0.4 ± 0.3
Brain	0.5 ± 0.4	0.6 ± 0.4	0.5 ± 0.2

In these studies, the rate of incorporation of acetate-2-^{14}C into sterols was taken as a measure of the inherent rate of synthesis for each tissue. It is possible, however, that in some tissues the rate of entry into the cell or the rate of activation of acetate to acetyl-CoA may be rate-limiting. This point was not evaluated in these studies.

Effect of Cholesterol Feeding and Fasting on Sterol Synthesis

Both cholesterol feeding and fasting are experimental procedures that are known to suppress hepatic cholesterogenesis markedly. In order to evaluate the effect of these two dietary manipulations upon sterol synthesis in extrahepatic tissues, we either placed groups of four animals on a high cholesterol diet for 6 wk or fasted them for 48 hr. The mean rates of incorporation of acetate-2-^{14}C into digitonin-precipitable sterols by slices of various organs of these two groups of animals are shown in columns B and C, respectively, of Table 1.

As expected, hepatic sterol synthesis was suppressed both by cholesterol feeding and by fasting. In extra-

TABLE 2 SYNTHESIS OF PARTICULAR STEROLS BY TISSUES OF CONTROL, CHOLESTEROL-FED, AND FASTED RATS

Values are mμmoles of acetate-2-^{14}C converted to total digitonin-precipitable sterols and to specific sterols per gram of tissue during a 2 hr incubation; the amount of ^{14}C-label found in each specific sterol is also expressed as a percentage of the counts found in the total digitonin-precipitable sterols in that particular tissue.

Tissue	Exptl. Condition of Animal	Total DP-Sterols	Cholesterol		Cholestanol		Methostenol (Δ^7 & Δ^8)		Δ^7-Cholestenol		Lanosterol	
				%		%		%		%		%
Liver	Control	171	152	(89)	3.4	(2)	5.1	(3)	1.7	(1)	3.4	(2)
		173	164	(95)	1.7	(1)	3.4	(2)	1.7	(1)	1.7	(1)
	Cholesterol-fed	2.5	2.1	(85)	<0.2		<0.2		<0.2		<0.2	
		1.0	0.8	(80)	<0.2		<0.2		<0.2		<0.2	
	Fasted	21	15.7	(75)	0.2	(1)	0.6	(3)	<0.2		0.6	(3)
		11	8.8	(80)	<0.2		0.2	(2)	<0.2		0.2	(2)
Ileum	Control	120	102	(85)	2.4	(2)	3.6	(3)	2.4	(2)	3.6	(3)
		89	73	(82)	0.9	(1)	3.6	(4)	1.8	(2)	3.6	(4)
	Cholesterol-fed	105	82	(78)	1.0	(1)	3.0	(3)	1.0	(1)	2.1	(2)
		125	90	(72)	1.2	(1)	2.5	(2)	1.2	(1)	3.7	(3)
	Fasted	82	62	(75)	0.8	(1)	1.6	(2)	0.8	(1)	3.3	(4)
		66	54	(82)	0.7	(1)	1.9	(3)	0.7	(1)	2.0	(3)
Transverse colon	Control	101	78	(77)	1.0	(1)	3.0	(3)	2.0	(2)	2.0	(2)
		52	43	(82)	1.5	(3)	2.1	(4)	1.6	(3)	1.6	(3)
	Cholesterol-fed	65	50	(77)	0.7	(1)	1.3	(2)	1.3	(2)	1.3	(3)
		73	53	(72)	1.4	(2)	1.4	(2)	2.9	(4)	2.9	(4)
	Fasted	47	34	(72)	0.5	(1)	1.4	(3)	0.9	(2)	1.9	(4)
		38	30	(80)	0.4	(1)	0.8	(2)	0.4	(1)	0.8	(2)
Stomach	Control	26	21.8	(84)	0.5	(2)	0.5	(2)	0.5	(2)	1.0	(4)
		36	32.6	(91)	0.4	(1)	0.7	(2)	0.7	(2)	0.7	(2)
	Cholesterol-fed	37	29.6	(80)	0.4	(1)	1.1	(3)	1.1	(3)	1.1	(3)
		41	34.0	(83)	0.4	(1)	0.8	(2)	0.8	(2)	1.6	(4)
	Fasted	29	22.3	(77)	0.3	(1)	1.2	(4)	0.9	(3)	0.6	(2)
		19	14.2	(75)	0.2	(1)	0.6	(3)	0.2	(1)	0.6	(3)
Jejunum	Control	10.2	9.0	(88)	0.3	(3)	0.5	(5)	0.3	(3)	0.3	(3)
		18.7	14.6	(78)	0.4	(2)	0.7	(4)	0.6	(3)	0.7	(4)
	Cholesterol-fed	9.5	8.1	(85)	0.4	(4)	0.7	(7)	0.3	(3)	0.2	(2)
		13.7	10.3	(75)	0.3	(2)	0.4	(3)	0.3	(2)	0.5	(4)
	Fasted	7.2	6.3	(87)	0.1	(2)	0.4	(6)	0.1	(2)	0.2	(3)
		5.0	4.2	(83)	0.2	(3)	0.3	(5)	0.2	(3)	0.2	(3)
Testis	Control	12.5	1.5	(12)	0.6	(5)	0.3	(2)	<0.1		6.5	(52)
		8.4	1.5	(18)	0.6	(7)	0.3	(3)	<0.1		4.9	(58)
	Cholesterol-fed	9.2	1.8	(20)	0.4	(4)	0.2	(2)	<0.1		3.9	(42)
		10.0	1.5	(15)	0.4	(4)	0.2	(2)	<0.1		5.9	(59)
	Fasted	7.6	1.4	(18)	0.5	(6)	0.1	(1)	<0.1		4.6	(60)
		11.1	3.0	(27)	0.3	(3)	0.2	(2)	<0.1		5.4	(49)
Lung	Control	6.8	4.3	(63)	0.1	(1)	0.2	(3)	<0.1		0.2	(3)
		6.6	3.8	(58)	0.1	(1)	0.1	(2)	<0.1		0.1	(2)
	Cholesterol-fed	5.0	3.1	(62)	<0.1		0.3	(6)	<0.1		0.3	(5)
		3.9	2.6	(67)	<0.1		0.1	(3)	<0.1		0.1	(3)
	Fasted	4.0	2.9	(72)	0.1	(2)	0.2	(4)	<0.1		0.2	(4)
		6.2	3.7	(59)	0.1	(1)	0.2	(3)	<0.1		0.2	(3)
Skin	Control	8.4	3.3	(40)	0.6	(7)	0.6	(7)	3.0	(36)	0.9	(10)
		5.2	1.9	(37)	0.3	(5)	0.5	(10)	1.4	(27)	0.8	(16)
	Cholesterol-fed	4.2	1.1	(26)	0.2	(5)	0.3	(8)	1.4	(34)	0.5	(11)
		6.9	2.7	(40)	0.6	(8)	0.5	(7)	1.5	(22)	0.6	(8)
	Fasted	2.0	0.6	(32)	0.1	(3)	0.1	(3)	0.6	(32)	0.1	(7)
		4.7	1.8	(39)	0.2	(5)	0.3	(7)	1.8	(39)	0.5	(10)

TABLE 2 *continued*

Tissue	Exptl. Condition of Animal	Total DP-Sterols	Cholesterol	Cholestanol	Methostenol (Δ^7 & Δ^8)	Δ^7-Cholestenol	Lanosterol
			%		%		%
Kidney	Control	2.3	2.0 (85)	<0.1	<0.1	<0.1	0.1 (4)
		1.7	1.2 (73)	<0.1	<0.1	<0.1	0.1 (3)
	Cholesterol-fed	4.1	2.7 (65)	<0.1	0.1 (2)	<0.1	0.1 (2)
		4.3	3.5 (81)	<0.1	0.1 (3)	<0.1	0.1 (2)
	Fasted	2.9	2.0 (68)	<0.1	<0.1	<0.1	0.1 (3)
		1.6	1.1 (73)	<0.1	<0.1	<0.1	<0.1
Spleen	Control	4.0	2.5 (64)	<0.1	<0.1	<0.1	0.2 (6)
		3.0	1.9 (62)	<0.1	<0.1	<0.1	0.2 (7)
	Cholesterol-fed	2.1	1.3 (62)	<0.1	<0.1	<0.1	0.1 (6)
		4.3	3.0 (70)	<0.1	<0.1	<0.1	0.3 (8)
	Fasted	1.9	1.3 (68)	<0.1	<0.1	<0.1	0.2 (10)
		1.7	1.3 (75)	<0.1	<0.1	<0.1	0.1 (8)

DP, digitonin-precipitable.

hepatic tissues, however, sterol synthesis was never significantly depressed in the cholesterol-fed or fasted animals.

These findings emphasize a remarkable difference between the biosynthetic pathways for sterol synthesis in hepatic and extrahepatic tissues. Clearly, only the liver responds to exogenous cholesterol and fasting, with a marked reduction in the rate at which sterols are made; synthesis in every other tissue goes on at virtually unaltered rates. As a consequence of this selective suppression, the ileum becomes the most active site for the incorporation of ^{14}C-acetate into digitonin-precipitable sterols in cholesterol-fed and fasted rats.

Specific Synthesis of Cholesterol in Control, Cholesterol-Fed, and Fasted Rats

In the experiments just described it was shown that sterol synthesis is depressed by cholesterol feeding and fasting only in the liver. However, since in these studies acetate-2-^{14}C incorporation was measured only in total digitonin-precipitable sterols, it was possible that the specific synthesis of cholesterol was suppressed in extrahepatic tissues but that this suppression was masked by the accumulation or overproduction of another labeled 3β-hydroxysterol. In order to examine this possibility, we separated the digitonin-precipitable fractions synthesized by different tissues into individual sterols by TLC and determined the relative ^{14}C-labeling of each of these sterols.

That digitonin precipitation results in a nearly quantitative recovery of sterols that are intermediates in the biosynthesis of cholesterol from the total unsaponifiable lipids is indicated by the data in Fig. 1. In this example the gas–liquid chromatographic pattern of the lipids present in the total unsaponifiable fraction of skin is compared with the pattern of the 3β-hydroxysterols precipitated from the same fraction as the digitonides. As is evident, the two patterns are nearly identical with respect to the relative areas of each of the sterol peaks. Only the peak that corresponds to lanosterol shows a decrease of about 50% in area (relative to the cholesterol–cholestanol peak) after digitonin precipitation of the sterols.

An example of the TLC of the digitonin-precipitable sterols of skin is illustrated in Fig. 2. After development in benzene–ethyl acetate 5:1, the tissue sterols are resolved into four areas that have the same R_f values as reference samples[1] of lanosterol, the methostenols (4α-methyl-Δ^7-cholestenol and 4α-methyl-Δ^8-cholestenol), cholesterol, and Δ^7-cholestenol. When each of these spots is scraped from the plate, eluted, and analyzed by GLC, the four patterns illustrated in Fig. 2 are obtained: spot 1 gives a single peak with the same retention time as lanosterol; spot 2 has two peaks whose retention times correspond to those of Δ^7- and Δ^8-methostenols; spots 3 and 4 correspond to cholesterol and Δ^7-cholestenol, respectively. In this TLC system cholestanol contaminates the cholesterol area, so that spot 3 must be rechromatographed on silver nitrate plates to separate the two sterols.

These TLC systems, therefore, make possible the separation of ^{14}C-labeled digitonin-precipitable sterols into five different fractions which are considered to be cholesterol, cholestanol, Δ^7-cholestenol, lanosterol, and a mixture of Δ^7- and Δ^8-methostenols. This identification has been confirmed by mass spectrographic analysis (kindly performed by Dr. E. Horning).

Table 2 depicts the results obtained when the digitonin-precipitable sterols synthesized by various tissues

[1] Kindly supplied by Doctors I. Franz and J. Wilson.

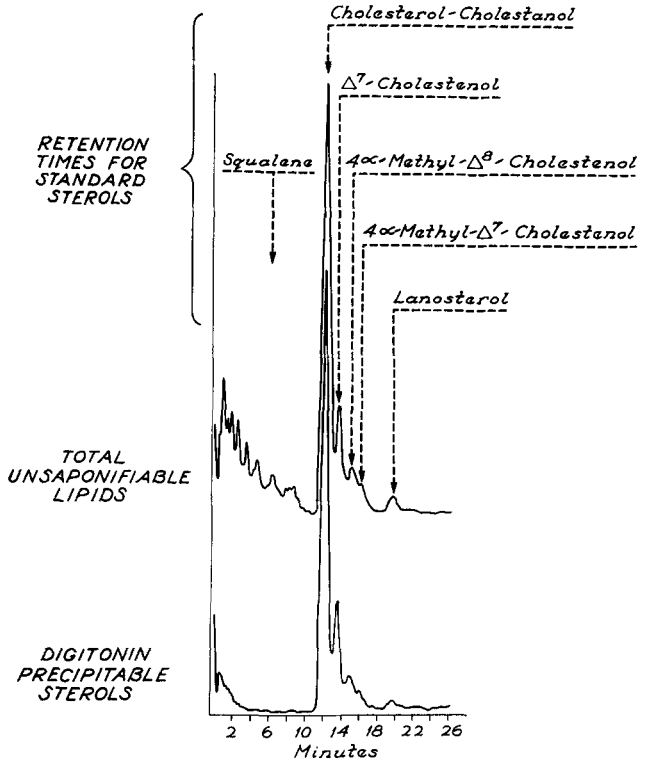

FIG. 1. Recoveries of various skin sterols by digitonin precipitation. Gas–liquid chromatograms (above) of the total unsaponifiable lipid fraction of skin and (below) of the digitonin-precipitable sterol fraction.

of the rat were fractionated. In these experiments, tissue slices of 10 different organs taken from two control animals, two animals fed a high cholesterol diet, and two fasted animals were incubated with labeled acetate. The first column shows the rates of acetate-2-^{14}C incorporation into total digitonin-precipitable sterols in each of these tissues. The last five columns give the ^{14}C-acetate incorporation rates into individual sterols; in addition, the percentage of ^{14}C counts in the total digitonin-precipitable fraction found in each of the specific sterol fractions is indicated in parentheses.

These data allow a definitive answer as to whether cholesterol synthesis in any extrahepatic tissue is inhibited by prolonged cholesterol feeding or fasting. The data presented in the second column show that hepatic cholesterogenesis was depressed about 100-fold by cholesterol feeding and nearly 11-fold by fasting. In contrast, in none of the nine extrahepatic tissues examined was comparable suppression of cholesterol synthesis found; indeed, the rates of cholesterogenesis in the organs of the cholesterol-fed animals were identical with those of respective organs in the control animals. Similarly, fasting resulted in either no depression or only slight reduction (2-fold at most) of cholesterol synthesis in the extrahepatic tissues. It thus appears that only the liver possesses the rate-controlling mechanisms that can strongly suppress sterol synthetic activity in the face of a high cholesterol intake or prolonged fasting.

DISCUSSION

Publications from several laboratories have reported in detail different aspects of sterol metabolism in several extrahepatic tissues; however, since the methodology has varied among the different studies, it is impossible to use these data for a quantitative comparison of the relative rates of cholesterogenesis in these various organs. The initial data obtained in the present investigation and reported in column A of Table I show that every tissue of the rat tested was capable of making sterols from acetate, yet the relative synthetic rates varied as much as 200-fold between different organs. In the control animals the highest rate of sterol synthetic activity was found in the liver. Synthesis in the gastrointestinal tract varied markedly depending upon the area examined: the acetate incorporation rate was low in the esophagus, duodenum, and jejunum, moderately high in the stomach and colon, and maximal in the terminal small bowel. Indeed, sterol synthesis in the ileum occurred at a rate second only to that found in the liver. The remaining viscera of the abdominal and thoracic cavities synthesized sterols at relatively low rates, while the tissues that manifested the lowest rate of synthesis included the three types of muscle (smooth, striated and cardiac) and the brain.

The relative importance of sterol synthesis in a given organ in the intact animal depends not only upon the synthetic rate in that tissue, but also upon the fraction of the total body weight represented by that particular organ system. For purposes of comparison, therefore, mean synthetic rates shown in column A of Table I have

TABLE 3 CALCULATED STEROL SYNTHESIS BY WHOLE TISSUES OF A 250 G RAT

Tissue	Acetate Converted to: A. Total DP-Sterols	B. Cholesterol
	mμmoles/2 hr	
Liver	1450	1330
Small bowel	600	530
Skin	190	70
Colon and cecum	150	120
Stomach	60	52
Skeletal muscle	53	—
Testis	21	3
Lungs	15	9
Kidneys	7	6
Spleen	2	1
Heart	0.5	—
Brain	0.5	—
Whole gastrointestinal tract	810	702

DP, digitonin-precipitable.

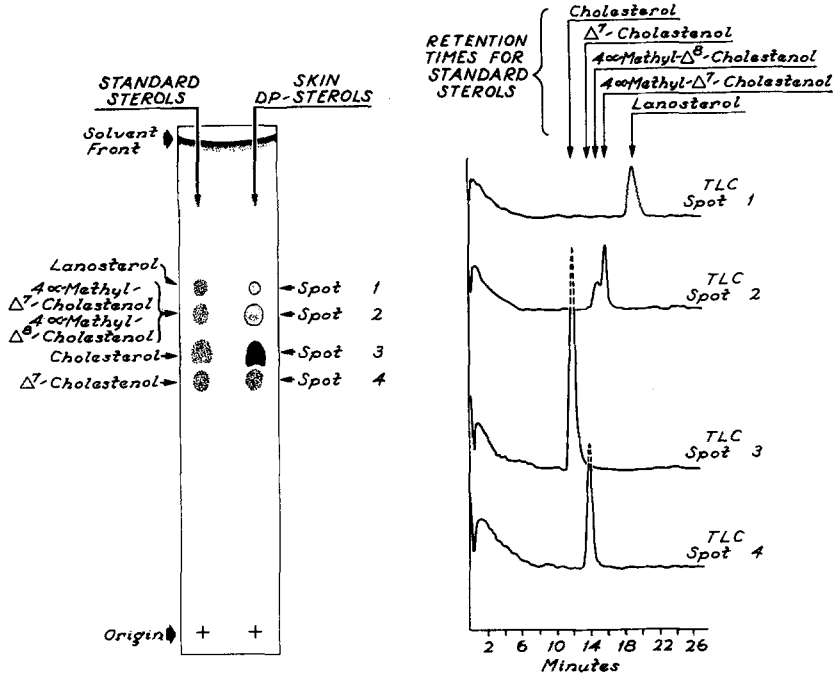

FIG. 2. TLC of digitonin-precipitable skin sterols. Left, the chromatoplate (developed in benzene–ethyl acetate 5:1). Right, GLC of the four spots obtained.

been multiplied by the respective mean whole organ weights for each of these tissues to give the potential "whole organ" synthesis of digitonin-precipitable sterols. In addition, from the percentage of the labeled 3β-hydroxysterols that is cholesterol, one can also calculate the potential "whole organ" rate of cholesterogenesis. The results of these calculations are presented in Table 3. Liver has the highest potential for both total digitonin-precipitable sterol and cholesterol synthesis, while the small bowel, again, ranks second. Synthetic activity in the whole gastrointestinal tract amounts to about 55% of that found in the liver; together, liver and gastrointestinal tract account for nearly 90% of the total synthetic activity found in the entire animal (exclusive of the skeletal system and bone marrow). Of the remaining organs, only skin and striated muscle appear to be significant sites of synthesis, but together they account for only about 9% of the total demonstrable whole body synthesis.

While these data emphasize which organ systems have the greatest potential for sterol synthetic activity, they do not indicate what proportion of this sterol may be used for structural purposes in formation of new cells and what amount, if any, may be contributed to the circulating cholesterol pool. It is of interest, however, that liver and intestine, the two organs found in this study to have the highest in vitro synthetic rates, have both been implicated as major contributors to the circulating blood sterol pool (3–6, 17, 20).

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