In Vivo Demonstration of the Cholesterol Feedback System by Means of a Desmosterol Suppression Technique

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ABSTRACT This report describes a "desmosterol suppression" technique with which it has been possible to demonstrate the operation of the cholesterol negative feedback system in the intact animal. 0.1% triparanol in the diet causes a virtually complete block in the conversion of desmosterol to cholesterol by liver and intestine. Since desmosterol is not consumed in the diet, the level of plasma desmosterol can be employed as an index of endogenous sterol production and release into the bloodstream. With this technique it was shown that the feeding of cholesterol for 8 days to rats decreases blood desmosterol levels to less than 5% of control values. Very similar results were obtained when cholesterol synthesis was assayed in vivo with acetate-^{14}C as a cholesterol precursor. These observations indicate that the cholesterol feedback system operates very effectively in the intact animal in suppressing the endogenous contribution to the circulating cholesterol pool. Since intestinal cholesterol synthesis is only slightly inhibited by exogenous cholesterol, these results also indicate that the intestine does not represent a significant source of plasma sterols in the rat.

INTRODUCTION

While the extensive in vitro studies of the past 20 yr have contributed greatly to our knowledge of the tissue sites (1, 2) and mechanisms of control of cholesterol synthesis at the cellular level (3), studies of cholesterogenesis using more physiologic, in vivo techniques, have proved far less definitive. It has, for example, been well established by isotopic methods that under in vitro conditions, cholesterol synthesis in the livers of all higher animals (4–7), including man (8), is under sensitive feedback control; however, the quantitative effect of exogenous cholesterol on cholesterol production when examined in vivo has yielded more variable results[1] (9–14). Moreover, while it is well-known that the intestine contributes to the plasma cholesterol pool (15, 16), the quantitative importance of this source of blood cholesterol in the intact animal remains to be established firmly.

Motivated in part by these questions as well as by the need to evaluate the in vivo effect of tumors upon the feedback control of cholesterol synthesis—the results of which are described in the following study (17)—we have attempted to develop a simple, nonisotopic procedure for assessing the approximate rates of sterol production and release into plasma in the intact animal. The technique described in this report estimates endogenous sterols in plasma by determining the level of plasma desmosterol in animals treated with triparanol, a drug that blocks cholesterol synthesis at the point of conversion of desmosterol to cholesterol (18, 19).

The results obtained with this method demonstrate that cholesterol feeding causes a virtually complete disappearance of endogenous sterols from the plasma, a finding which indicates that the cholesterol feedback system operates very effectively in the intact rat. These data also suggest that at least in this species, the intestine, while actively synthesizing sterols, represents a relatively minor source of *plasma* sterols.

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[1] Wilson, J. D. The relation between cholesterol absorption and cholesterol synthesis in the baboon. Submitted for publication.

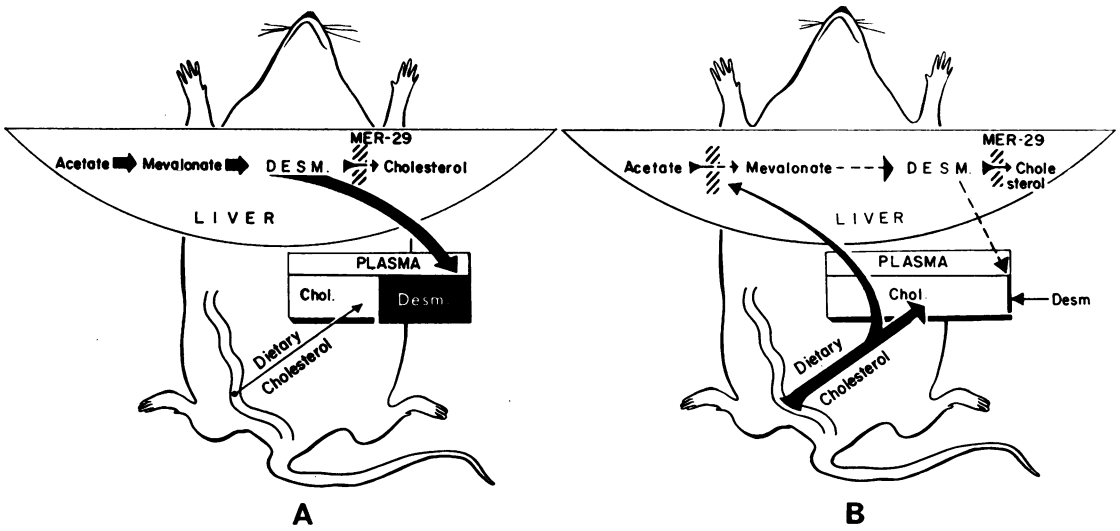

FIGURE 1 Rationale for the use of triparanol to demonstrate cholesterol feedback control in vivo. Triparanol-fed rats given either low cholesterol (A) or high cholesterol (B) diets are schematically shown.

METHODS

Animals. Rats used in this study were young adult females of the ACI/f Mai[2] or Buffalo[3] strains and weighed between 150 and 250 g. Ground B & D Mastermade Rat Cubes[4] served as the basal diet throughout the study. The high cholesterol diet was made by adding 50 g of recrystallized (from ethanol) cholesterol, dissolved in 500–700 ml of ethyl ether, to 1 kg of ground rat pellets. After thoroughly mixing the feed for 5–10 min, the ether was evaporated at room temperature. Triparanol[5] was added as a powder to the test diets to give a concentration of 0.1% by weight. It should be noted that during the time period that this diet was employed, the rats remained healthy and lost no weight.

Analytical procedures. Desmosterol and cholesterol were measured by saponifying 0.1 ml of whole blood, 0.5 ml of lymph or weighed amounts of tissues with 15 vol of ethanolic KOH (1 part 10 N KOH + 2 part absolute ETOH), for 1 hr at 37°C (blood and lymph) or 2 hr at 100°C (tissue). The nonsaponifiable sterols were extracted with petroleum ether, dissolved in acetone, and a portion injected directly onto a 6 or 12 ft gas-liquid chromatographic (GLC)[6] column, OV-17,[7] 1%, on 100–120 mesh gas chrom Q. An internal standard of tritiated cholesterol was incorporated into each sample to permit correction for losses during the isolation of the sterols. A Barber-Colman model 10 gas-liquid chromatograph equipped with an argon-ionization detector was used for all GLC analyses. With a column temperature of 221°C and a gas flow of 100/ml per min, the retention times of cholesterol and desmosterol were 18 and 22 min respectively. Areas of the chromatographic curves were determined by planimetry. Desmosterol was obtained from Steraloids, Inc., Pawling, N. Y., and gave a single peak on gas chromatography; the detector responses for cholesterol and desmosterol were identical. In labeling experiments, ^{14}C in the gas efflux was collected on toluene-moistened cotton using a Packard sample collector (Packard Instrument Co.) equipped with a subambiant temperature head cooled with dry ice and isopropanol, and was counted in a Beckman liquid scintillation counter, using *p*-bis[2-(5-phenyloxazolyl)]benzene ("POPOP"), 2,5-diphenyloxazole ("PPO") in ethyl acetate: toluene, 6.6: 93.4 as the scintillation solution. Total and esterified sterols were determined by the Sperry-Webb procedure (20). Desmosterol by this method gives a color yield approximately 60% that of cholesterol.

In vitro studies were carried out exactly as described previously (21), cholesterol and desmosterol being isolated by GLC as noted above. In vivo incorporation of acetate-2-^{14}C into sterols was determined by injecting 50 μCi of acetate-2-^{14}C intraperitoneally into rats 2 hr before killing. Rats were exsanguinated by heart puncture under light ether anesthesia. The organs to be studied were saponified, the sterol digitonides isolated and their total ^{14}C content determined as previously described (21) or analyzed by gas-liquid chromatography as noted above. In the studies of release of sterols into the lymph, intestinal lymph was collected using the method of Weis and Dietschy (22).

RESULTS

Rationale. The rationale of the procedure reported in this and the following paper is illustrated in Fig. 1 A and 1 B.

It is well established that both fasting (23) and exogenous cholesterol (21, 24) depress the rate of hepatic cholesterol synthesis in the liver by inhibiting the reactions responsible for the conversion of β-hydroxy-β-

[2] Microbiological Associates, Inc., Bethesda, Md.
[3] Simonson Laboratories, San Francisco, Calif.
[4] B & D Mills, Grapevine, Tex.
[5] The triparanol (MER/29) used in these studies was kindly furnished by Dr. Carl A. Bunde, Director of Medical Research, The W. S. Merrell Company, Cincinnati, Ohio.
[6] *Abbreviation used in this paper:* GLC, gas-liquid chromatography.
[7] Applied Science Laboratories, Inc., State College, Pa.

TABLE I

Blood Desmosterol Concentrations in Rats Fed Low Cholesterol Diets, Low Cholesterol Diets with Triparanol, and High Cholesterol Diets with Triparanol

	Desmosterol concentration	
Low cholesterol	Low cholesterol and triparanol	5% cholesterol and triparanol
	mg/100 ml	
<0.25	7	<0.25
<0.25	4	<0.25
<0.25	10	<0.25
<0.25	4	<0.25
<0.25	4	<0.25
Mean <0.25	6 ±1	<0.25

methylglutarate to mevalonate. It would follow that if a unique intermediate in cholesterol synthesis such as desmosterol that is produced beyond the point of mevalonate synthesis could be accumulated and measured in the bloodstream, the rate of appearance of such a compound should give an approximation of the rate of mevalonate synthesis.

Effect of cholesterol feeding on desmosterol levels in blood. To evaluate this approach of estimating sterol synthesis in the intact rat, the effect on plasma desmosterol levels of inhibiting hepatic sterol synthesis with exogenous cholesterol was determined in the intact, triparanol-treated rat. Rats were first placed on either low cholesterol diets,[8] or diets containing 5% cholesterol, and 2 or 3 days later triparanol (0.1% by weight) was added to the diets of the experimental animals. Over a subsequent 7–14 day period retrobulbar blood samples were taken from each rat for desmosterol analysis. The results of GLC analyses of blood sterols on the 8th day after initiation of triparanol feeding are shown in Fig. 2. As illustrated in Fig. 1 B, if this approach is valid, one would expect that dietary cholesterol would, by blocking mevalonate synthesis in the liver, lead to a marked suppression of the level of blood desmosterol in the triparanol-treated animals.

As shown by the gas chromatographic analysis illustrated in Fig. 2 A, in the normal, untreated rat on a low cholesterol diet, cholesterol represents the major sterol detected in the blood. After treatment with triparanol for 8 days, the animal on a low cholesterol diet develops a very significant desmosterol peak (Fig. 2 B). Most important, however, as shown in Fig. 2 C, the feeding of a high cholesterol diet to a triparanol-treated rat causes a virtually complete suppression of plasma desmosterol.

[8] The commercial diets fed these rats contained 0.072% cholesterol.

The actual levels of blood desmosterol in these three groups of rats are shown in Table I. No desmosterol was detected in the blood of the normal control rat. After 8 days of triparanol treatment, blood desmosterol reached concentrations of 3–10 mg/100 ml, i.e. levels of desmosterol that constitute about 10–30% of the total blood sterol concentration. By contrast, however, in the five rats fed the high cholesterol diet, blood desmosterol concentrations were markedly reduced to those of the rats fed the triparanol-free diet. Subsequent studies have demonstrated that the limit of detection of desmosterol by this gas-liquid chromatographic procedure is approximately 0.25 mg/100 ml; and that, moreover, this figure in fact is the approximate concentration of desmosterol actually present in the blood of the cholesterol-fed, triparanol-treated rats in this study.

A more extensive examination of the effect of dietary cholesterol on blood desmosterol levels is shown in Fig. 3. In this study desmosterol suppression was examined in two species of rats, the Buffalo and ACI/f Mai, over a 14 day period of triparanol feeding. The results conform to those in Table I; however, in this experiment the values for blood desmosterol were consistently higher in both the low and high cholesterol-fed rats than were those in the earlier study. It is nonetheless apparent that the exogenous cholesterol resulted in a marked suppression in blood desmosterol levels in both of the species of rats examined. It is particularly noteworthy that in this experiment the concentration of desmosterol continues to rise beyond the 8 day period of triparanol feeding routinely used throughout these studies. As a result, when carried beyond the 8 days of triparanol feeding, the difference in desmosterol concentration between the two groups of animals increases. This experiment therefore suggests that the degree of suppression of desmosterol in the blood of cholesterol-fed rats can readily be amplified by extending the period during which the animals are fed the experimental diets.

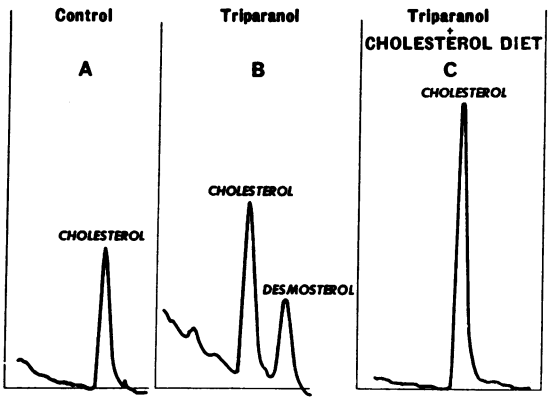

FIGURE 2 GLC analysis of cholesterol and desmosterol in blood of normal, triparanol, and cholesterol-fed rats.

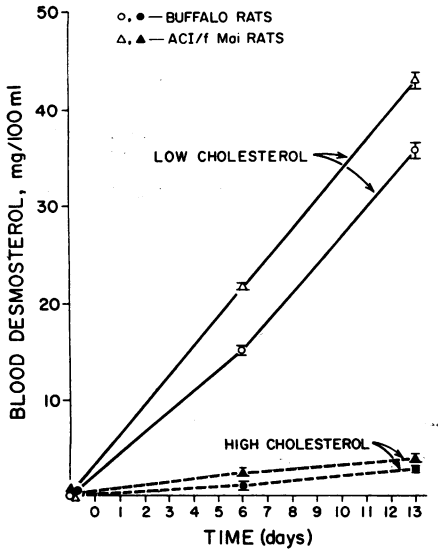

FIGURE 3 Effect of prolonged triparanol and cholesterol feeding upon blood levels of desmosterol in two strains of rats. Each group of animals consisted of four rats. The ranges shown represent the mean ±SEM.

The effect of cholesterol feeding on the conversion of acetate-2-^{14}C to cholesterol in vivo. To provide experimental support for the validity of employing blood desmosterol levels as a measure of endogenous sterol synthesis, studies were performed to determine the effect of cholesterol feeding upon the synthesis of sterols from acetate-^{14}C in the triparanol-treated rat. The design of the study was identical with that in the previous section except that 2 hr before sacrifice, the rats were injected intraperitoneally with 50 μCi of acetate-^{14}C,[9] and the incorporation of ^{14}C into total digitonin precipitable sterols, i.e. cholesterol, desmosterol, and other sterols, in the liver and blood was determined. As the results in Table II demonstrate, the feeding of cholesterol causes a marked reduction in the incorporation of acetate-2-^{14}C into the sterols of both the liver and the plasma. This effect is, moreover, observed in both normal and triparanol-treated animals. It is apparent, therefore, that the use of desmosterol levels in the plasma as an indication of relative sterol synthesis will yield results that are comparable with those obtained with isotopic techniques.

In vitro synthesis of cholesterol and desmosterol in liver. In order to provide further evidence that the synthesis of desmosterol represents an accurate index of endogenous sterol production, the effect of dietary cholesterol upon hepatic desmosterol and cholesterol production was determined in vitro. Rats were placed on either the high or low cholesterol diets for a total of 11 days, and one-half of each group of animals was treated with triparanol for the last 8 days of the study. At the end of this period 350 mg of liver slices were incubated with 10 μCi sodium acetate-2-^{14}C, and total digitonin-precipitable sterol-^{14}C, desmosterol, and cholesterol, as well as desmosterol-^{14}C and cholesterol-^{14}C were determined as described under "Methods."

The results shown in Table III closely mirror those observed in the intact animals. The analysis of the nonradioactive sterols demonstrate that desmosterol represents a major component of the sterol fraction in the livers of the triparanol-fed rats on the low cholesterol diet; the feeding of cholesterol, however, again caused an almost complete disappearance of this hepatic desmosterol as detected by gas-liquid chromatography.

As expected, the in vitro incorporation of acetate-2-^{14}C into total digitonin-precipitable sterols was markedly inhibited by the high cholesterol diet, digitonin-precipitable sterol-^{14}C being reduced to from 0.5 to 3% of that produced in the livers of the animals on the low cholesterol diet. Moreover, this suppression of total hepatic sterol synthesis was also obtained after cholesterol feeding in animals that were treated with triparanol.

Determination of the ^{14}C in the individual sterols isolated by GLC demonstrated that after cholesterol feeding, the triparanol-treated animals incorporated acetate-^{14}C

TABLE II
Isotopic Demonstration of Cholesterol Feedback Control In Vivo

Triparanol in diet	Cholesterol in diet	Sterol-^{14}C (nmoles acetate-^{14}C incorporated/g of tissue or ml of plasma) in: Plasma	Liver	Intestine
%				
		1.1	6.8	9.6
None	Low	1.0	6.5	10.5
		1.2	7.9	7.9
	Average	1.1	7.1	9.3
		0.02	0.06	4.7
None	High	0.03	0.09	3.5
		0.03	0.07	7.2
	Average	0.03	0.07	5.1
		1.2	17.1	10.2
0.1	Low	1.1	9.2	7.2
		0.7	11.6	9.5
	Average	1.0	12.6	9.0
		0.02	0.10	2.3
0.1	High	0.02	0.07	2.6
		0.03	0.12	3.1
	Average	0.02	0.10	2.7

[9] Sodium acetate-2-^{14}C, 2 μCi/μmole, was purchased from New England Nuclear Corp., Boston, Mass.

TABLE III

Feedback Inhibition of Cholesterol and Desmosterol Synthesis in Liver In Vitro

Rat	Triparanol % in diet	Cholesterol in diet	Plasma sterol*	Hepatic sterol concentration: Total sterol*	Free sterol*	Ester sterol*	Desmosterol‡	Hepatic sterol synthesis acetate-2-^{14}C incorporated into: Dig. Ppt. sterols§	Cholesterol	Desmosterol	Other sterols
			mg/100 ml		*mg/g*			*nmoles/g per 2 hr*		*cpm*	
1	0	Low	74	2.38	2.21	0.17	—	127	3,060	180	228
2	0	Low	75	2.11	1.63	0.48	—	71	2,300	215	335
3	0.1	Low	28	1.58	1.58	—	0.2	207	290	3,860	1,500
4	0.1	Low	36	1.83	1.74	0.09	0.5	112	130	1,600	1,365
5	0	High	247	26.4	2.91	23.5	—	—	175	105	370
6	0	High	313	25.7	8.38	17.7	—	2	5	5	3
7	0.1	High	156	35.6	5.20	30.4	<0.001	2	120	90	380
8	0.1	High	237	22.8	3.19	19.6	<0.001	1	—	—	—

* Liebermann Burchard Reacting Sterols read at 35 min. The approximate error of this method is 12% at the desmosterol concentration of about 20% present in the liver and with a comparable error of 7% for plasma; no corrections are incorporated in the figures.

‡ Analyzed by gas-liquid chromatography.

§ Digitonin-precipitable sterols.

into the GLC peak corresponding to desmosterol to an extent only 5% of that observed in the rats fed the low cholesterol diet. In comparable samples from livers of rats not treated with triparanol, the same inhibition of sterol synthesis was seen; however, the radioactivity under these circumstances appeared in the cholesterol peak rather than in the desmosterol fraction. It is noteworthy that the data in Table III also indicate that triparanol in the dose employed in this study does not significantly inhibit total hepatic sterol synthesis but in both experiments (rats 3 and 4) caused a greater than 90% block in the conversion of desmosterol to cholesterol by the liver.

In a similar in vitro study, the ^{14}C in the various sterol fractions was determined by continuously assaying the ^{14}C during the GLC separation of the sterols synthesized by the liver of normal and triparanol-treated rats. As the combined GLC and ^{14}C analysis of these sterols shown in Fig. 4 A illustrates, in the absence of triparanol the majority of ^{14}C is incorporated into cholesterol with small but significant activity being present in the area corresponding to desmosterol. A similar analysis from an animal treated with triparanol, Fig. 4 B, shows an almost complete absence of ^{14}C in cholesterol, while the desmosterol peak and to a lesser extent at least three unidentified compounds with no detectable mass by GLC, contain all of the recovered radioactivity. This study again demonstrates that treatment with triparanol effectively stops the synthesis of cholesterol; and under these conditions, endogenous cholesterol is almost completely replaced by newly synthesized desmosterol. Finally, in Fig. 4 C are shown the results of such an analysis from a triparanol-treated rat fed the high cholesterol diet. Again, it is apparent that exogenous cholesterol effectively suppresses the desmosterol peak noted in Fig. 4 B; however, equally significant, the dietary cholesterol has caused a complete block in the synthesis of both cholesterol and desmosterol with the result that detectable ^{14}C is no longer incorporated into either of these sterol fractions.

Sources of endogenous plasma sterols. The degree of suppression of endogenous blood sterols observed after cholesterol feeding was somewhat unexpected since on the basis of previous studies it was assumed that, while the liver is responsible for supplying a major fraction of plasma sterols, extrahepatic tissues such as the intestine (14, 16, 27) might be expected to make a significant contribution to this sterol pool.

Three possible explanations for these findings could be postulated. It was quite possible that (*a*) triparanol might not penetrate the intestinal cell and so would not block the conversion of desmosterol to cholesterol in this tissue or, (*b*) even were the triparanol present within the intestinal cell, it was conceivable that the intestine might normally make use of a biosynthetic pathway of sterol synthesis that does not involve desmosterol synthesis. (*c*) It was possible that triparanol might inhibit the release of sterols into the lymph and, as a result, this desmosterol could not reach the plasma. The first two of these three possibilities could be excluded by the following series of experiments.

Cholesterol and desmosterol synthesis in slices of intestine obtained from triparanol-treated animals. To determine the effect of triparanol upon sterol synthesis in the intestine, the conversion of acetate-2-^{14}C to desmosterol and cholesterol was examined in intestinal slices obtained from triparanol-fed rats. Animals were fed either the normal stock diet or a 5% cholesterol diet while experimental rats were given the same diets to which was added triparanol in a concentration of 0.1%.

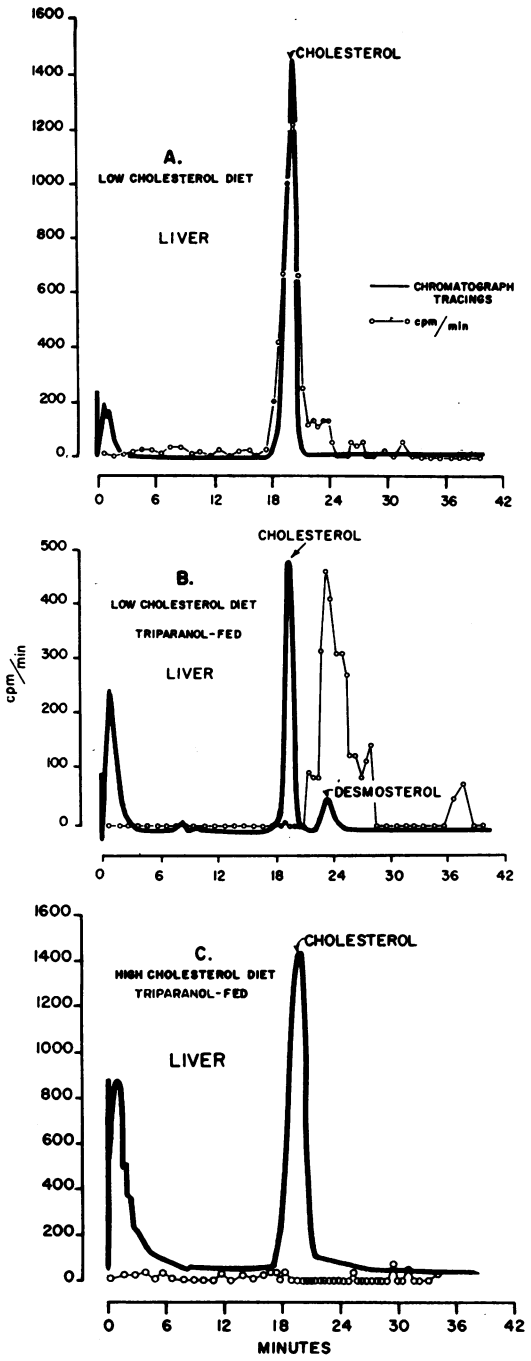

FIGURE 4 Simultaneous measurement of sterols and in vitro incorporation of acetate-^{14}C into sterols in livers of rats fed: A, normal diet; B, triparanol; and C, triparanol and a high cholesterol diet.

After 8 days of triparanol feeding, the animals were killed and slices from the ileum were assessed for their ability to synthesize desmosterol from acetate-2-^{14}C, as described under "Methods." As indicated by the data in Table IV, under such in vitro conditions even in the absence of triparanol significant quantities of ^{14}C are incorporated into desmosterol and other digitonin-precipitable sterols. Treatment with triparanol resulted in a 95% inhibition of cholesterol synthesis with a comparable *increase* in ^{14}C incorporation into desmosterol. As a result the *total* digitonin-precipitable sterols synthesized in the intestine were not influenced by the triparanol treatment.

As has been previously noted (2, 7), after prolonged cholesterol feeding some decrease in sterol synthesis is observed in the intestine presumably due to a bile acid mediated feedback inhibition of mevalonate production (25, 26); however, it is apparent that in the cholesterol-fed animal, too, triparanol treatment leads to a block in the conversion of desmosterol to cholesterol in the intestine.

It may be concluded, therefore, that cholesterol synthesis in the intestine, as in the liver, can proceed by way of desmosterol; and that triparanol both enters the intestinal cell, and there causes a virtually complete inhibition in the conversion of desmosterol to cholesterol.

Release of endogenous sterols into intestinal lymph. The influence of triparanol upon the secretion of newly synthesized sterols into the lymph was next studied. Two triparanol-(0.1%) fed rats and two control animals were maintained on the low cholesterol diet for 2 days. Cannulae were then placed in the intestinal lymph ducts, and after satisfactory lymph flow was apparent, i.e. 10–90 min, 50 μCi acetate-2-^{14}C was injected intraperitoneally. The recovery of ^{14}C in the digitonin-precipitable sterols of the lymph over the following 24 hr is shown in Table V. These data demonstrate that triparanol does not significantly alter the incorporation of acetate-^{14}C into the sterols of intestinal lymph.

The effect of a high cholesterol diet upon desmosterol levels in the intestinal lymph of triparanol-treated animals was next examined. In this study one pair of rats was placed on the high cholesterol and another pair on the low cholesterol diet for 11 days. Both groups of animals were given triparanol 0.1% in their diets during the final 8 days of the study. On the last day intestinal lymph was collected for 24 hr and analyzed for cholesterol and desmosterol as described under "Methods." The results shown in Fig. 5A demonstrate that desmosterol represents a major lymphatic sterol in the triparanol-treated rats on the low cholesterol diet; by contrast, cholesterol feeding, while causing the concentration of lymphatic cholesterol to rise significantly, results in an almost total disappearance of desmosterol from the lymph, Fig. 5B.

These two experiments, therefore, suggest that the intestine releases small amounts of newly synthesized sterols into the intestinal lymph and hence into the sys-

TABLE IV

Effect of Triparanol and Cholesterol upon Intestinal Sterol Synthesis In Vitro

Diet	Triparanol	Total digitonin precipitable sterol-^{14}C (nmoles acetate-^{14}C converted/g intestine per 2 hr)	Cholesterol	Desmosterol	Other sterols
				*cpm**	
Low cholesterol*	−	152	2,250	1,645	2,815
Low cholesterol§	−	8.3	585	566	800
Low cholesterol§	−	23.6	1,638	1,097	908
Low cholesterol§	−	10.7	783	870	483
Low cholesterol‡	+	262	100	3,670	2,695
Low cholesterol§	+	—	53	1,421	592
Low cholesterol§	+	—	15	419	523
High cholesterol‡	−	71	170	245	405
High cholesterol§	−	9.8	887	668	—
High cholesterol‡	+	27	25	320	730

* "cpm" represents total counts per minute collected from the gas chromatograph effluent during appearance of the cholesterol and desmosterol peaks. "Other sterols" refers to all other ^{14}C collected before and for 10 min after the desmosterol peak. Equal portions of the digitonin-precipitable sterols were injected onto the columns and the counts per minute therefore provide a measure of the relative synthesis of cholesterol and desmosterol.

‡ Incubated with 5.0 μmoles acetate-2-^{14}C.

§ Incubated with 2.0 μmoles acetate-2-^{14}C.

temic circulation; moreover, they indicate that treatment with triparanol in the doses employed does not significantly inhibit the release of such endogenous sterols into the lymph.

DISCUSSION

The primary purpose of the present study was to establish the feasibility of employing desmosterol concentrations in the blood of triparanol-treated animals as a means of assessing the contribution of endogenous sterols to the blood cholesterol pool. The rationale of this approach assumes that, since desmosterol is normally not present in the diet, if this sterol is released into the plasma and metabolized at approximately the same rate as is cholesterol, then desmosterol should serve as an easily measurable, nonisotopic, biological label for endogenous sterols. The validity of this method has been established in the present study in several ways. First, it could be demonstrated that as measured by acetate-^{14}C incorporation the in vitro synthesis of desmosterol in the livers of triparanol-treated rats re-

TABLE V

Failure of Triparanol to Affect the Output of Labeled Sterols into Lymph

Triparanol in diet	Cumulative total ^{14}C in digitonin precipitable sterols at:		
	4 hr	12 hr	24 hr
%		*cpm*	
0	6,310	16,050	24,690
0.1	7,230	15,960	18,780

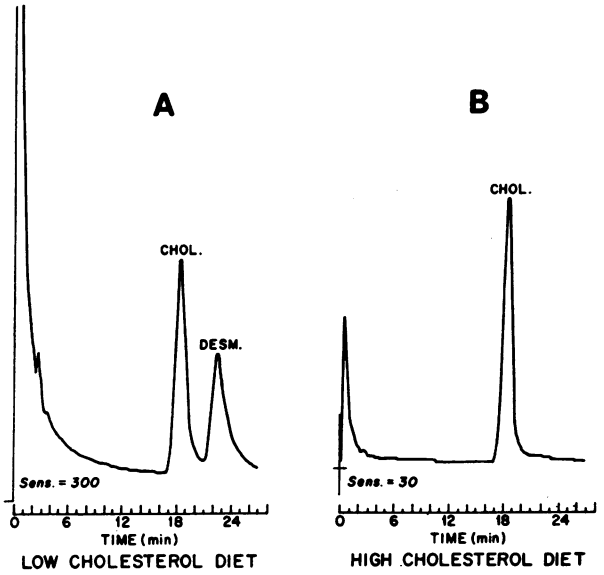

FIGURE 5 Effect of cholesterol feeding on lymph desmosterol levels in triparanol-treated animals. Note that the detector sensitivity of the chromatograph from the cholesterol-fed rat is one-tenth that of the animal fed the low cholesterol diet.

flects the rates of synthesis of total sterols or of cholesterol in untreated animals. Secondly, it was shown that in the dose employed, triparanol does not influence the over-all rate of sterol synthesis, but does cause an almost complete block in the conversion of desmosterol to cholesterol in both of the tissues, i.e. the liver and the intestine, that are believed to contribute significantly to the circulating sterol pool. Thirdly, by the use of acetate-^{14}C to assess sterol synthesis in vivo, it could be established that both the total and labeled desmosterol present in the plasma accurately parallels the appearance of newly synthesized cholesterol in the circulation.

Taken together, these data strongly support the validity of employing plasma desmosterol concentrations as a means of estimating endogenous sterol levels in the bloodstream. Obviously, the accumulation of a cholesterol precursor such as desmosterol at any single point in time can provide only a relative, not an absolute, value for sterol synthesis; moreover, this estimate will also be influenced by any factors which cause variations in the sterol turnover rate. On the other hand, as shown in this study, feeding 0.1% triparanol causes the almost complete replacement of endogenous cholesterol by desmosterol. In theory, therefore, the rate of appearance of desmosterol in the blood of triparanol-treated animals should then provide turnover data comparable with those obtained with radioactive sterol precursors.

During the course of these studies, several observations of physiologic significance have been made regarding the sources of plasma cholesterol and the effectiveness of the cholesterol feedback system in the intact animal. While the liver is known to be a major source of plasma cholesterol, the intestinal mucosa is capable of very active cholesterol synthesis (1, 2, 6, 25, 26), and can readily release such synthesized sterols into the plasma via the intestinal lymph (15, 16). Moreover, dietary cholesterol does not influence the rate of secretion of this newly synthesized cholesterol into lymph (27). The quantitative importance of the intestinal contribution to plasma cholesterol has been difficult to assess accurately; however, most investigators have concluded that in the rat (12, 13), and probably to a greater extent in higher species (10, 14, footnote 1, 16), extrahepatic tissues contribute significantly to the circulating sterol pool. Related to this problem is the question of the completeness with which exogenous cholesterol can inhibit cholesterol synthesis in the intact animal. In contrast to the case of liver, intestinal cholesterol synthesis is relatively insensitive to inhibition by exogenous cholesterol (6, 20, 25). It then follows that if the intestine represents a major source of plasma cholesterol, dietary cholesterol might effectively suppress hepatic cholesterogenesis, yet the over-all effect of exogenous cholesterol in suppressing the endogenous cholesterol contributed to plasma cholesterol would be minimal. The results of the present study using plasma desmosterol levels and isotope incorporation into cholesterol as indications of endogenous sterol production indicate that the feeding of cholesterol causes at least a 90% suppression of endogenous sterols in the plasma; and we would conclude, therefore, that cholesterol feedback control of the endogenous plasma sterol pool is extremely effective in the intact rat.

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REFERENCES

1. Srere, P. A., I. L. Chaikoff, S. S. Treitman, and L. S. Burstein. 1950. The extrahepatic synthesis of cholesterol. *J. Biol. Chem.* **182**: 629.
2. Dietschy, J. M., and M. D. Siperstein. 1967. Effect of cholesterol feeding and fasting on sterol synthesis in seventeen tissues of the rat. *J. Lipid Res.* **8**: 97.
3. Siperstein, M. D. 1970. Regulation of cholesterol biosynthesis in normal and malignant tissues. *In* Current Topics in Cellular Regulation. B. L. Horecker and E. R. Stadtman, editors. Academic Press, Inc., New York. **2**: 65–100.
4. Gould, R. G. 1951. Lipid metabolism and atherosclerosis. *Amer. J. Med.* **11**: 209.
5. Tomkins, G. M., H. Sheppard, and I. L. Chaikoff. 1953. Cholesterol synthesis by liver. III. Its regulation by ingested cholesterol. *J. Biol. Chem.* **201**: 137.
6. Gould, R. G., C. B. Taylor, J. S. Hagerman, I. Warner, and D. J. Campbell. 1953. Cholesterol metabolism. I. Effect of dietary cholesterol on the synthesis in dog tissue *in vitro*. *J. Biol. Chem.* **201**: 519.
7. Dietschy, J. M., and J. D. Wilson. 1968. Cholesterol synthesis in squirrel monkey: relative rates of synthesis in various tissues and mechanisms of control. *J. Clin. Invest.* **47**: 166.
8. Bhattathiry, E. P. M., and M. D. Siperstein. 1963. Feedback control of cholesterol synthesis in man. *J. Clin. Invest.* **42**: 1613.
9. Eckles, N. E., C. B. Taylor, D. J. Campbell, and R. G. Gould. 1955. The origin of plasma cholesterol and the rates of equilibration of liver, plasma, and erythrocyte cholesterol. *J. Lab. Clin. Med.* **46**: 359.
10. Taylor, C. B., D. Patton, N. Yogi, and G. E. Cox. 1960. Diet as source of serum cholesterol in man. *Proc. Soc. Exp. Biol. Med.* **103**: 768.
11. Harper, P. V., W. B. Neal, and G. R. Hlavacek. 1953. Lipid synthesis and transport in the dog. *Metab. (Clin. Exp.)*. **2**: 69.
12. Morris, M. D., I. L. Chaikoff, J. M. Felts, S. Abraham, and N. O. Fansah. 1957. The origin of serum cholesterol in the rat: diet versus synthesis. *J. Biol. Chem.* **224**: 1039.
13. Jansen, G. R., M. E. Zanetti, and C. F. Hutchison.

1966. Studies on lipogenesis *in vivo*. Effects of starvation and re-feeding and studies on cholesterol synthesis. *Biochem. J.* **99**: 333.
14. Wilson, J. D., and C. A. Lindsey, Jr. 1965. Studies on the influence of dietary cholesterol on cholesterol metabolism in the isotopic steady state in man. *J. Clin. Invest.* **44**: 1805.
15. Lindsey, C. A., Jr., and J. D. Wilson. 1965. Evidence for a contribution by the intestinal wall to the serum cholesterol of the rat. *J. Lipid Res.* **6**: 173.
16. Wilson, J. D. 1968. Biosynthetic origin of serum cholesterol in the squirrel monkey: evidence for a contribution by the intestinal wall. *J. Clin. Invest.* **47**: 175.
17. Bricker, L. A., H. P. Morris, and M. D. Siperstein. 1972. Loss of the cholesterol feedback system in the intact hepatoma-bearing rat. *J. Clin. Invest.* **51**: 206.
18. Avigan, J., D. Steinberg, M. J. Thompson, and E. Mosettig. 1960. Mechanism of action of MER-29, an inhibitor of cholesterol biosynthesis. *Biochem. Biophys. Res. Commun.* **2**: 63.
19. Avigan, J., D. Steinberg, H. E. Vroman, M. J. Thompson, and E. Mosettig. 1960. Studies of cholesterol biosynthesis. The identification of desmosterol in serum and tissues of animals and man treated with MER-29. *J. Biol. Chem.* **235**: 3123.
20. Sperry, W. M., and M. Webb. 1950. A revision of the Schoenheimer-Sperry method for cholesterol determination. *J. Biol. Chem.* **187**: 97.
21. Siperstein, M. D., and M. J. Guest. 1960. Studies on the site of the feedback control of cholesterol synthesis. *J. Clin. Invest.* **39**: 642.
22. Weis, H. J., and J. M. Dietschy. 1969. Failure of bile acids to control hepatic cholesterogenesis: evidence for endogenous cholesterol feedback. *J. Clin. Invest.* **48**: 2398.
23. Bucher, N. L. R., P. Overath, and F. Lynen. 1960. β-hydroxy-β-methyl glutaryl coenzyme A reductase, cleavage and condensing enzymes in relation to cholesterol formation in rat liver. *Biochim. Biophys. Acta.* **40**: 491.
24. Siperstein, M. D., and V. M. Fagan. 1966. Feedback control of mevalonate synthesis by dietary cholesterol. *J. Biol. Chem.* **241**: 602.
25. Dietschy, J. M., and M. D. Siperstein. 1955. Cholesterol synthesis by the gastrointestinal tract: localization and mechanisms of control. *J. Clin. Invest.* **44**: 1311.
26. Dietschy, J. M. 1968. The role of bile salts in controlling the rate of intestinal cholesterogenesis. *J. Clin. Invest.* **47**: 286.
27. Wilson, J. D., and R. T. Reinke. 1968. Transfer of locally synthesized cholesterol from intestinal wall to intestinal lymph. *J. Lipid Res.* **9**: 85.