Loss of the Cholesterol Feedback System in the Intact Hepatoma-Bearing Rat

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ABSTRACT By means of the desmosterol suppression technique described in the previous paper, the influence of hepatomas on sterol metabolism has been studied in the intact rat. The major finding of this study is that all hepatoma-bearing rats demonstrate a consistent in vivo loss of the cholesterol feedback system that is characteristic of normal liver. The results also demonstrate that such tumors retain only minor amounts of the sterol they synthesize, releasing over 90% of such endogenous sterol into the circulation. Finally, the in vivo loss of cholesterol feedback control was found to occur in at least two minimal deviation hepatomas and in one highly malignant adenocarcinoma of hepatic origin. These findings indicate that even tumors that are capable of only very limited cholesterol synthesis in vitro, can contribute significant quantities of sterol to the bloodstream.

It is concluded that as a result of their lack of normal cholesterol feedback control, hepatomas may represent a physiologically important source of sterols in the tumor-bearing animal, and that the absence of feedback control of sterol synthesis may provide a means of detecting the presence of such tumors in the intact animal.

INTRODUCTION

It is now well established that cholesterol synthesis is regulated in the livers of higher animals through a sensitive negative feedback system (1–4) by which dietary cholesterol specifically inhibits those reactions in the cholesterol biosynthetic pathway responsible for the conversion of β-hydroxy-β-methylglutarate to mevalonate (5, 6). We have previously reported that in striking contrast to the consistency with which this feedback mechanism can be demonstrated in normal liver, all hepatomas of man, rodents, and fish examined to date show a complete absence of feedback control of cholesterol synthesis (7–12), and of β-hydroxy-β-methylglutaryl CoA reductase (13), the enzyme responsible for mevalonate synthesis. Cholesterol synthesis in such hepatomas is, therefore, no longer suppressable by dietary cholesterol and as a result, these tumors produce relatively large amounts of mevalonate and, in many cases, of cholesterol as well.

It should be noted, however, that the above conclusions have been based entirely upon in vitro studies in which cholesterol synthesis was measured in tissue slices or homogenates obtained from either normal livers or from tumors. In view of the fact that the absence of cholesterol feedback control in hepatomas remains the only known example of the consistent loss of a normal feedback system in any tumor, it seemed important to establish, first, whether absence of this feedback system could be detected in the intact tumor-bearing animal, and second, whether the sterols produced by tumors as a result of this feedback defect are retained within the tumor cells for structural purposes or, alternately, whether such sterols are released into the bloodstream to contribute to circulating sterol pools.

In the preceding study (14), it was demonstrated that while inhibition of sterol synthesis by the drug triparanol causes a marked accumulation of desmosterol in the blood of rats fed a normal diet, the feeding of cholesterol results in an almost complete suppression of plasma desmosterol. This demonstration of the pres-

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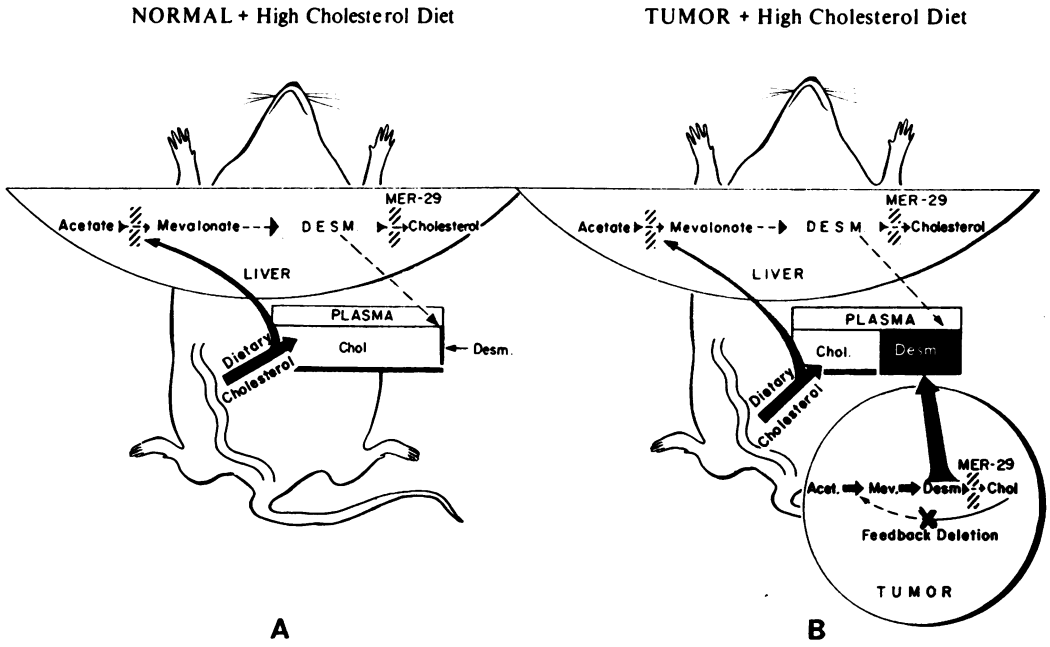

FIGURE 1 Rationale for use of triparanol to demonstrate loss of the cholesterol feedback system in hepatoma-bearing, high cholesterol-fed rats in vivo.

ence of the cholesterol feedback system under in vivo conditions in the normal animal suggested the possibility that the absence of the cholesterol feedback system might be detected by a similar technique in the intact, tumor-bearing animal.

The results reported here clearly demonstrate that primary liver tumors readily release newly synthesized sterols into the circulation. Moreover, the finding that, in the presence of a hepatoma, exogenous cholesterol fails to inhibit *de novo* sterol production provides the first in vivo demonstration of the loss of the cholesterol feedback system in the tumor-bearing animal.

METHODS

The methods of feeding cholesterol and triparanol, and of administering acetate-^{14}C, follow the protocol described in the previous study (14). The details of the method employed for determining plasma and liver desmosterol and cholesterol levels are likewise described in the preceding paper (14).

The hepatomas employed in this study were all originally supplied by one of the authors (H. P. M.). The highly differentiated hepatoma 7787, the well differentiated 9121, and the poorly differentiated hepatoma 3924A have been maintained through repeated implantations at the University of Texas for approximately 4 yr.

Hepatoma 7787 was implanted subcutaneously in Buffalo rats, while hepatomas 3924A and 9121 were grown intramuscularly in the ACI/f Mai rat strain. Further details of the experimental procedures are described under the individual studies.

RESULTS

In order to evaluate both the in vivo synthesis of sterols in hepatomas as well as the possible release of such endogenous sterols into plasma, use was made of the concept developed in the preceding paper, that in the triparanol-treated animal, the level of desmosterol in the plasma provides an approximate measure of endogenous sterol production and subsequent release into the circulation.

The rationale of this approach as it was applied to the tumor-bearing rat is schematically shown in Fig. 1. On the basis of the in vitro demonstration of the consistent absence of cholesterol feedback control in hepatomas (7, 8), one would expect that cholesterol feeding would not affect desmosterol production by hepatomas in vivo. If in fact hepatomas are capable of synthesizing *desmosterol,* and if this desmosterol can in turn be released into plasma, it would be anticipated that in the presence of such a tumor, plasma desmosterol would remain elevated even after complete suppression of hepatic desmosterol synthesis by dietary cholesterol.

Desmosterol accumulation in cholesterol-fed, triparanol-treated rats bearing hepatoma 7787. Fig. 2 represents typical gas-liquid chromatographs from an experiment designed to test this hypothesis. In Fig. 2 A, B, and C are shown the results of sterol analyses in normal rats treated with a normal diet, 0.1% triparanol for 8 days, or triparanol plus 11 days of cholesterol feeding.

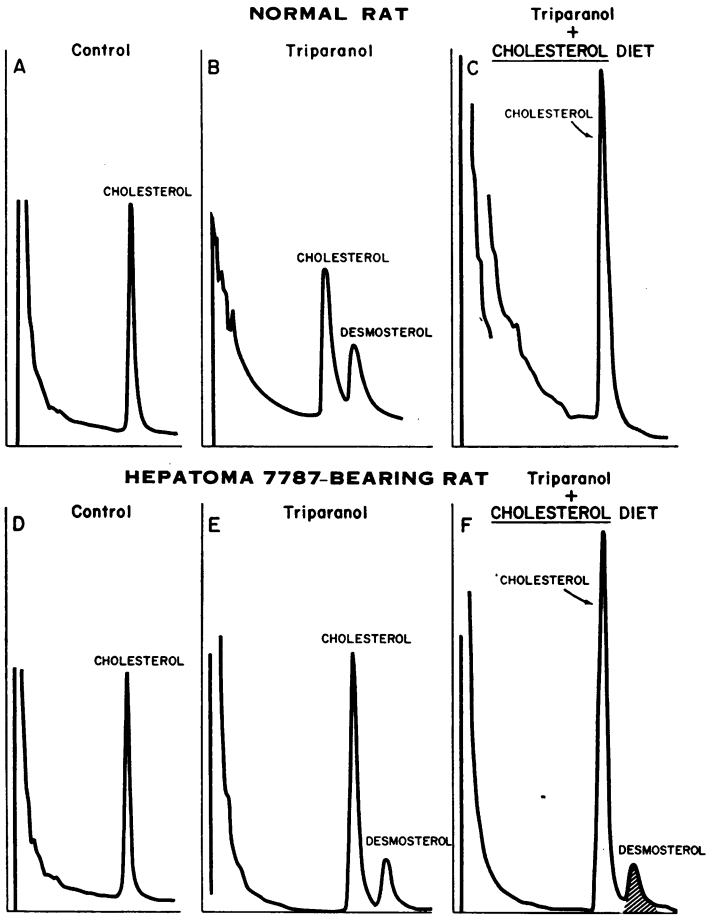

FIGURE 2 GLC demonstration of feedback control of sterol synthesis in normal rats (A,B,C); and loss of the cholesterol feedback system in rats bearing hepatoma 7787 (D,E,F).

These chromatograms are similar to those presented in the previous study (14), and serve again to demonstrate the almost complete disappearance of plasma desmosterol (Fig. 2 C) when cholesterol is fed to a triparanol-treated normal rat.

In Fig. 2 D, E, and F are shown chromatograms obtained from comparable rats implanted with hepatoma 7787. The results are strikingly different from those seen in the tumor-free animal. Fig. 2 D illustrates the single cholesterol peak found in most tumor-bearing rats fed a normal diet in the absence of triparanol. As shown in Fig. 2 E, triparanol causes an accumulation of blood desmosterol in the tumor-bearing animal just as was observed in the normal rat. However, most significantly, in contrast to the almost total suppression of plasma desmosterol produced by dietary cholesterol in the normal rat, Fig. 2 C, the desmosterol levels of the tumor-bearing rat. Fig. 2 F, were unaffected by the high cholesterol diet.

The actual desmosterol concentrations in the blood of the tumor-bearing and control animals, are shown in Table I. The feeding of triparanol to normal animals results in desmosterol concentrations which in these studies averaged 7 mg/100 ml, while in the hepatoma-bearing rats, a significantly higher desmosterol concentration of 14 mg/100 ml was achieved on triparanol feeding. The results of administering a high cholesterol diet to the normal and tumor-bearing rats again demonstrate the disappearance of desmosterol from the blood of the normal rat. This finding, however, contrasts strikingly with the observation that, in the tumor-bearing animal, cholesterol feeding is completely incapable of suppressing the concentration of plasma desmosterol (14 mg/100 ml) below that of the animal fed the low cholesterol diet.

These data, therefore, clearly demonstrate that desmosterol synthesis persists in the hepatoma-bearing animal despite complete feedback suppression of normal hepatic cholesterogenesis.

Loss of the cholesterol feedback system in the pres-

ence of the minimal deviation hepatoma 9121. Evidence that the deletion of the cholesterol feedback system can be demonstrated in vivo in hepatomas other than the highly differentiated hepatoma 7787 is presented in Fig. 3. In this study a second well-differentiated hepatoma, 9121, which we have previously (8) demonstrated by in vitro techniques to have lost the cholesterol feedback system, was studied in vivo. The design of the study was identical with that used to examine the hepatoma 7787 except that samples of blood were obtained at three intervals during the study. As demonstrated by the dashed line in Fig. 3, feeding cholesterol to four normal, triparanol-treated rats totally prevented the appearance of desmosterol in the plasma (< 0.25 mg/100 ml) during the entire 7.5 day period of study. By contrast, in four animals bearing the 9121 tumor (solid line in Fig. 3), desmosterol continued to accumulate in the blood and reached levels averaging 93 mg/100 ml.

These data therefore demonstrate that at least two different varieties of hepatomas will cause the loss of the cholesterol feedback system in the intact animal.

In vivo feedback deletion produced by adenocarcinoma 3924A. Studies were next carried out to determine whether the presence of a highly malignant, poorly differentiated tumor, which is known to synthesize cholesterol only at very low rates, will also result in detectable loss of the cholesterol feedback system in vivo. One of the most malignant of the hepatic tumors originally produced by treating rats with diacetoaminofluorine is the 3924A hepatoma, which is a highly undifferentiated adenocarcinoma having few characteristics of normal liver (16), and synthesizes cholesterol at only minimal rates (8). It was surprising, therefore, to find that (as shown in Fig. 4) after 7 and 11 days of triparanol feeding, the desmosterol concentrations of the blood of rats bearing the 3924A tumor averaged at least three times that of the control rats, which in this experiment had low but significant concentrations of desmosterol in their blood. These data indicate that when present in the intact animal for many days, even a very poorly differentiated tumor will cause a blunting of the cholesterol feedback response.

TABLE I

Blood Desmosterol Levels in Normal and Hepatoma 7787–Bearing Rats

Desmosterol Concentration mg/100 ml ±SE

Control	Triparanol	Triparanol + cholesterol diet
Normal rat		
<0.1	7	<0.1
<0.1	2	<0.1
<0.1	10	<0.1
Average <0.1	7 ±2	<0.1
Hepatoma 7787–bearing rat		
<0.1	14	7
<0.1	12	18
<0.1	14	16
<0.1	15	12
Average <0.1	14 ±0.6	14 ±3

Synthesis of desmosterol by hepatoma 9121 in vitro. In view of the demonstration in the preceding study

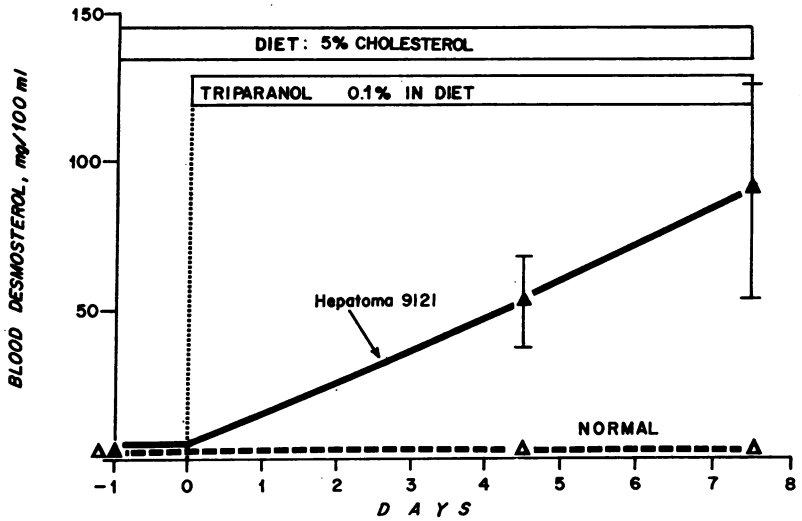

FIGURE 3 Time course of blood desmosterol accumulation in normal and hepatoma 9121–bearing rats. Each group of animals consisted of four rats. The ranges shown represent the mean ±SEM.

TABLE II

Feedback Inhibition of Cholesterol and Desmosterol Synthesis

Triparanol in diet	Choles. in diet	Tumor weight	Plasma sterol	Hepatic sterol concentration				Hepatic sterol synthesis: acetate-2-^{14}C incorporated into:			
				Total choles.*	Free choles.*	Ester choles.*	Desmosterol	Dig. ppt. sterols	Cholesterol	Desmosterol	Other sterols
%		g	mg/100 ml	mg/g	mg/g	mg/g	mg/g	nmoles/g per 2 hr	cpm	cpm	cpm
0	low	7.9	120	2.13	2.13	0	<0.003	49	755	120	275
0	low	11.9	173	2.33	1.76	0.57	<0.003	11	275	70	165
0.1	low	10.4	48	1.75	1.75	0	1.4	21	135	480	580
0.1	low	2.2	42	1.61	1.50	0.11	0.7	52	95	1,080	760
0	high	1.8	289	28.4	3.26	25.1	<0.003	2	170	40	330
0	high	17.1	235	51.2	9.42	41.8	<0.003	1	40	15	85
0.1	high	10.8	166	22.3	3.66	18.6	0.1	2	0	20	70
0.1	high	4.3	139	23.8	4.33	19.4	0.1	1	5	5	20

* Burchard-Liebermann reacting sterols read at 35 min. The approximate error of this method is 6% 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.

(14) that neither the liver nor the intestine contributes significant amounts of sterol to the circulation of such cholesterol-fed animals, it seems highly likely that the source of the blood desmosterol in such tumor-bearing rats is the hepatoma itself. To support this possibility, it was first necessary to demonstrate both that triparanol can actually enter the tumor cell and block the conversion of desmosterol to cholesterol in such cells. Slices of both liver and hepatoma 9121 obtained from rats that had previously been fed normal or cholesterol-containing diets with or without 0.1% triparanol were therefore examined for their ability to synthesize desmosterol and cholesterol from acetate-2-^{14}C, Table II.

Several incidental observations were made during the course of this study. First, it was noted that the livers of tumor-bearing rats synthesize digitonin-precipitable sterols at somewhat lesser rates than was observed in the livers of normal animals (Table III of reference 14),[1] a probable feedback response to the sterols produced by the tumor. Secondly, blood cholesterol levels in the hepatoma-bearing rats are clearly elevated (120 and 173 mg/100 ml) above those seen in normal rats (Table III of reference 14), an observation that further supports the conclusion that the tumors release quantitatively important amounts of endogenous sterols into the circulation. This conclusion is supported by the fact that the livers of the triparanol-fed, tumor-bearing rats accumulate large amounts of desmosterol, even when hepatic desmosterol synthesis is completely suppressed by dietary cholesterol.

It is also noteworthy from the data in Table II that the feeding of triparanol does not itself cause a decrease in total sterol synthesis by the liver despite the fact, clearly shown by these data, that the drug produces a virtually complete block in the hepatic conversion of desmosterol to cholesterol. The data in Table II also indicate that dietary cholesterol inhibits the hepatic incorporation of acetate into cholesterol in the tumor-bearing rat; and in a similar rat treated with triparanol this feedback inhibition leads to a marked depression of hepatic desmosterol synthesis as well.

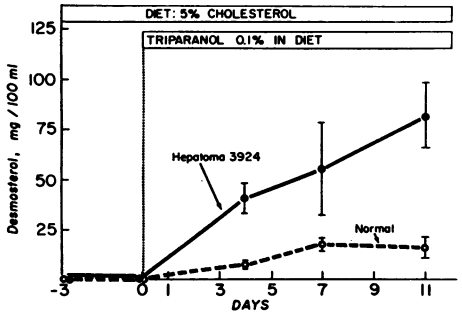

FIGURE 4 Loss of sterol feedback control in vivo in the presence of a malignant hepatoma 3924. Each group of animals consisted of four rats. The ranges shown represent the mean ±SEM.

[1] As noted earlier, cholesterol feeding has no detectable effect upon desmosterol levels in the tumor-bearing rat, a finding that would suggest an almost complete feedback inhibition of hepatic sterol synthesis in the tumor-bearing rat. The discrepancy between this conclusion and the 75% suppression of cholesterol synthesis actually observed in such livers is probably not significant in view of the relatively large variation in the plasma desmosterol levels found in these hepatoma-bearing rats.

in Liver and Hepatoma 9121 In Vitro

Tumor sterol concentration				Tumor sterol synthesis acetate-2-^{14}C incorporated into:			
Total choles.*	Free choles.*	Ester choles.*	Desmosterol	Dig. ppt. sterols	Cholesterol	Desmosterol	Others sterol
mg/g	*mg/g*	*mg/g*	*mg/g*	*nmoles/g per 2 hr*	*cpm*	*cpm*	*cpm*
2.82	1.88	0.94	0.003	328	8,340	1,530	2,630
2.36	1.12	1.24	0.003	184	5,500	1,330	1,730
1.93	2.02	—	1.6	195	40	1,705	1,805
1.76	—	—	1.0	178	105	2,865	2,435
2.89	1.92	0.97	0.003	183	—	—	—
2.67	1.69	0.98	0.003	169	9,380	1,130	1,495
2.20	2.29	—	2.6	308	115	7,215	4,915
1.84	1.47	0.37	1.6	270	175	5,350	3,200

Most important, however, it is apparent that hepatoma 9121 is capable of converting acetate-2-^{14}C to desmosterol at least as effectively as is liver. These data therefore indicate that the pathway of cholesterol synthesis in hepatomas is, in this respect, similar to that of normal liver and that triparanol does penetrate the hepatoma cell, and there causes at least a 95% inhibition in the conversion of desmosterol to cholesterol. Finally, this in vitro study clearly demonstrates that, in contrast to the response of normal liver, both desmosterol and cholesterol synthesis in the hepatoma is totally resistant to feedback inhibition by exogenous cholesterol, e.g., average desmosterol-^{14}C of 2285 cpm on the low cholesterol diet vs. 6282 on the high cholesterol diet.[2]

Further studies on the source of desmosterol in tumor-bearing rats. While the results of both the previous experiments indicate that the desmosterol present in the plasma of the tumor-bearing rats is derived from the tumor, since previous in vitro studies have demonstrated that minimal deviation hepatomas readily synthesize mevalonate even in the presence of a high cholesterol diet (8), it was conceivable, as illustrated in Fig. 5, that this mevalonate might be released into the bloodstream, carried to the liver, and there converted into desmosterol. It was therefore important to determine whether the plasma desmosterol detected in the tumor-bearing rats fed the high cholesterol diet was derived directly from the tumor itself or indirectly by way of the liver.

To examine this problem, use was made of the fact that after cholesterol feeding is continued for longer than 4 days, *hepatic* cholesterogenesis becomes inhibited at a point beyond the synthesis of mevalonic acid (5,10, 17,18). If prolonged cholesterol feeding could be shown to inhibit the conversion of mevalonate to cholesterol in the liver of a *tumor-bearing rat,* it would be highly unlikely that the desmosterol found in the plasma of such animals could be derived from the liver.

As demonstrated in Table III, after the feeding of cholesterol for 5 days to rats bearing hepatoma 9121,

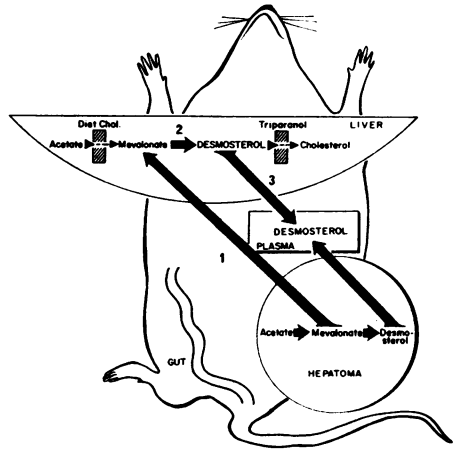

FIGURE 5 Possible sources of plasma desmosterol in a hepatoma-bearing rat.

[2] During GLC significant ^{14}C was observed in compounds with both earlier and later retention times than those of cholesterol and desmosterol. These probably represent "early labeled" cholesterol precursors (15), but were not further studied.

TABLE III

Effect of 5 Days of Cholesterol Feeding on Mevalonate Conversion to Cholesterol in Livers of Rats Bearing Hepatoma 9121

	Hepatic cholesterol synthesis from:	
	Acetate	Mevalonate
	nmoles/g liver per 2 hr	
Low cholesterol	67	46
	98	46
	69	41
	—	—
Average	78 ±7.7	44 ±2.0
High cholesterol	0.4	5.6
	0.2	11.8
	0.3	5.8
Average	0.3 ±0.06	7.7 ±2.0

the conversion of mevalonate to cholesterol decreases to 17% of the control levels, despite which the synthesis of cholesterol in the 9121 hepatoma itself continues unimpeded (8). It is apparent therefore that by the 8th day of cholesterol feeding, the time period routinely used in the previous in vivo studies, the livers of the tumor-bearing animals would not be capable of producing the large quantities of desmosterol present in the plasma.

Isotopic confirmation of loss of the cholesterol feedback system in vivo. In order to confirm by an independent method that hepatomas cause the in vivo loss of the cholesterol feedback system, the influence of hepatomas on cholesterol feedback control was assessed by employing the incorporation of parenterally administered acetate-^{14}C into plasma cholesterol as the measure of endogenous sterol synthesis and release. Rats with implanted 9121 hepatomas were fed triparanol and cholesterol exactly as described in the previous studies. On the day of the experiment the rats received 50 μCi of acetate-2-^{14}C intraperitoneally, and 2 hr later they were exsanguinated under light ether anesthesia. Total digitonin-precipitable sterol-^{14}C, which includes both cholesterol-^{14}C and desmosterol-^{14}C, was then determined in the blood and liver as previously described (5).

The results presented in Table IV demonstrate that significant amounts of labeled cholesterol and desmosterol are present in the blood of the triparanol-treated animals fed a normal diet; and, as was also shown in the previous study (14), the feeding of cholesterol causes a marked decrease in the appearance of this newly synthesized sterol fraction in both the plasma and the liver.

In contrast to the results in normal rats, in animals implanted with the hepatoma 9121, the feeding of a high cholesterol diet causes little if any depression in the amount of newly synthesized sterols released into the plasma. As shown in Table IV, the ^{14}C recovered in the total digitonin-precipitable fractions in either the presence or absence of triparanol was quite comparable regardless of whether the animal was on a low or high cholesterol diet. This failure of dietary cholesterol to inhibit the appearance of plasma sterol-^{14}C in the tumor-bearing rat was, moreover, observed at a time when the incorporation of acetate-^{14}C into liver sterols was greatly depressed by the cholesterol feeding.

Finally, as shown in Table IV, the large amounts of sterol-^{14}C recovered in the tumors themselves serve to demonstrate the very rapid synthesis of sterols within the tumor and confirms findings (7, 8), that in contrast to its effect in liver, dietary cholesterol does not depress sterologenesis in hepatomas.

DISCUSSION

In a series of studies employing various isolated tissue systems, this laboratory has previously documented the fact that the cholesterol feedback system, which controls cholesterol synthesis in normal liver, is consistently absent in a wide variety of hepatomas (7–11), as well as in leukemic cells.[3] Despite the success of the in vitro approach to this problem, it has been apparent that the demonstration of the loss of the cholesterol feedback system in an intact animal would be important for at least two reasons. First, if the absence of this feedback system could be demonstrated by a simple in vivo technique, a means might be at hand for detecting the presence of hepatomas and perhaps of other tumors in the intact animal; and second, it would be of physiologic interest to determine whether such tumors, having lost their cholesterol feedback control mechanism, autonomously release significant quantities of newly synthesized sterols into the bloodstream.

The present study makes use of two separate in vivo techniques to demonstrate that, in fact, hepatomas cause the loss of the cholesterol feedback system in the intact animal. As shown by the data in Table IV, the incorporation of injected acetate-2-^{14}C into plasma sterols can be used to demonstrate the operation of the cholesterol feedback system in the intact, normal animal; by contrast, in the hepatoma-bearing rat the incorporation of acetate-^{14}C into plasma sterols is far less affected by exogenous cholesterol. Hence, by this isotopic approach, hepatomas can clearly be shown to result in the loss of feedback control of the circulating cholesterol pool in the intact animal.

[3] Siperstein, M. D., E. M. Nadel, L. J. Luby, and J. R. Britton. Deletion of the cholesterol negative feedback system in leukemic cells. In preparation.

TABLE IV

Isotopic Demonstration of Hepatoma-Induced Loss of Cholesterol Feedback Control In Vivo

Treatment	Condition	Cholesterol in diet	Sterol-^{14}C (nmoles acetate-2-^{14}C incorporated/ g of tissue or ml of plasma) in: Plasma	Liver	Tumor	Intestine
None	Normal	Low	0.18	1.3		6.1
			0.93	5.8		10.3
			0.60	4.0		8.9
			0.57	*3.7*		*8.4*
	Normal	High	0.02	0.2		2.2
			0.03	0.2		6.4
			0.03	0.3		3.5
			0.03	*0.2*		*4.0*
	Hepatoma 9121	Low	0.10	0.6	10.4	4.5
			1.67	7.6	5.1	5.5
			0.51	1.7	26.1	3.7
			0.76	*3.3*	*13.9*	*4.6*
	Hepatoma 9121	High	0.17	0.08	16.9	1.3
			0.15	0.05	5.3	1.5
			0.34	0.13	20.0	2.0
			0.22	*0.09*	*14.1*	*1.6*
Triparanol	Normal	Low	0.28	4.4		7.6
			0.92	11.7		11.5
			0.96	11.5		17.6
			0.72	*9.2*		*12.2*
	Normal	High	0.01	0.3		2.1
			0.03	0.3		5.9
			0.03	0.5		1.9
			0.02	*0.4*		*3.3*
	Hepatoma 9121	Low	0.96	1.2	22.4	5.2
			0.23	0.8	12.7	6.1
			0.12	0.5	11.8	4.1
			0.44	*0.8*	*15.6*	*5.1*
	Hepatoma 9121	High	0.14	0.2	23.0	3.4
			0.18	0.2	16.4	2.6
			0.13	0.2	21.7	2.2
			0.15	*0.2*	*20.4*	*2.7*

The italicized numbers represent the averages of the respective columns.

A second method of assessing the presence of the cholesterol feedback system under in vivo conditions has been more extensively studied in the preceding publication (14). This procedure employs the suppressibility of plasma desmosterol by dietary cholesterol in the triparanol-treated rat as a measure of cholesterol feedback control. In view of the sensitivity of the gas-liquid chromatographic method of detecting desmosterol levels, this nonisotopic technique has the added advantage of permitting the repeated, sequential assessment of cholesterol feedback control using blood samples as small as 0.1 ml. With this approach, too, it has been possible to demonstrate that hepatomas consistently lead to the loss of the cholesterol feedback system in the intact host animal. This breakdown of cholesterol feedback control has, moreover, been documented in rats with two different varieties of minimal deviation hepatoma; and perhaps most important, a highly malignant adenocarcinoma has also been shown to produce a comparable loss of the cholesterol feedback system. The latter finding suggests that such undifferentiated tumors, despite the fact that by in vitro assay they synthesize cholesterol only very poorly, must over the course of an 11 day period release significant quantities of newly synthesized sterols into the bloodstream.

Several lines of evidence indicate that the source of

the desmosterol found in the blood of tumor-bearing animals is in fact the tumor itself. First, studies of sterol synthesis in hepatoma slices demonstrated that this tumor makes cholesterol via a desmosterol intermediate, and that in the dose of triparanol employed in this study, this drug produces an almost complete suppression of desmosterol conversion to cholesterol in such tumors. As a result, in hepatomas, which normally synthesize significant amounts of cholesterol, desmosterol rather than cholesterol, becomes the major end product of sterol synthesis. Secondly, both this, and the previous study (14) demonstrate that the cholesterol feedback system is operative in the intact animal and that during cholesterol feeding neither the liver nor the intestine can contribute significant amounts of endogenous sterol to the blood. This fact, coupled with the finding that hepatomas have no feedback control, strongly suggests that the desmosterol regularly found in blood of the cholesterol-fed, *tumor*-bearing animal, must be derived from the tumor itself.

The release of large quantities of endogenous sterols into the bloodstream by hepatomas might well be expected to have a significant influence on the over-all sterol metabolism of the tumor-bearing animal. The most obvious effect of such a hepatoma is the significant elevation of plasma cholesterol that they consistently produced in the tumor-bearing rats (Table II; and Table III of reference 14). While the quantitative aspects of this abnormal source of plasma cholesterol have not been extensively explored in the present study, it is apparent that the endogenous sterols derived from the hepatoma must be released in quantities sufficient to exert a feedback inhibition upon cholesterol synthesis in the liver of the tumor-bearing animal. The first suggestive evidence of such an effect consisted of the finding (Table I) that cholesterol feeding caused no detectable decrease in the plasma desmosterol levels of tumor-bearing rats. This observation suggested that cholesterol derived from the hepatoma inhibits hepatic cholesterogenesis to the point that the addition of dietary cholesterol could cause no further suppression of endogenous sterol production.

Direct evidence that the sterols produced by hepatomas do in fact cause feedback suppression of hepatic cholesterol synthesis is provided by the in vitro data shown in Table II. In these studies the presence of tumors sharply inhibited the synthesis of both cholesterol and desmosterol in the liver. It should be noted that an identical observation has been made previously by McGarry and Foster (20). We have previously reported that exogenous cholesterol represents a far more effective inhibitor of hepatic cholesterol synthesis than is endogenous cholesterol (19); however, over long periods of time high levels of endogenous cholesterol, probably through enterohepatic circulation, lead to a severe inhibition of hepatic cholesterol synthesis. It is likely that through this mechanism the sterols produced over many months by tumors can cause the direct inhibition of hepatic cholesterol synthesis observed in the present experiments.

After triparanol feeding the livers of tumor-bearing animals contain large quantities of desmosterol even though desmosterol synthesis by the liver had been blocked by exogenous cholesterol; and it follows, therefore, that this hepatic desmosterol must have originated from the hepatoma. These findings indicate that the sterols synthesized by the tumor contribute significantly not only to the blood but to the hepatic sterol pool as well. A quantitative estimate of the relative amounts of endogenous sterols secreted into the plasma can be obtained by assuming a total sterol turnover rate in an adult rat of approximately 6–7 mg/day (21). Since, as suggested above, the vast majority of the endogenous sterol in a hepatoma-bearing rat is derived from the tumor, it follows that even if the tumor does not cause an increase in the sterol turnover rate, during the period of at least 6 months during which this hepatoma is visible a minimum of 1080 mg (180 days × 6 mg/day) of sterol must be released primarily by the tumor into the circulation. In the course of this period of time, a 10 g hepatoma with an average sterol content of 2 mg/g tissue would have retained only approximately 20 mg of sterol. Even by gross approximation, therefore, it is apparent that the bulk of the sterol produced by the hepatoma must have been released into the blood rather than retained by the tumor.

It should incidentally be noted that despite the fact that triparanol treatment (as indicated by the data in Table II) causes a virtually complete block in cholesterol synthesis in both the tumor and the liver, tumor growth in the triparanol-treated animals proceeds at approximately the same rate as in animals not receiving triparanol. Apparently therefore desmosterol can substitute effectively for cholesterol in providing functional cell membranes in such tumors.

Finally, from a practical standpoint, the desmosterol suppression technique provides a means of detecting hepatomas while they are still of relatively small size.[4] Moreover, the finding that the 3924 hepatic adenocarcinoma responds similarly to well-differentiated liver

[4] There is already a precedent for employing desmosterol levels for the detection of certain tumors. Paoletti and coworkers (22, 23), have noted that some glial tumors of the brain may secrete sterols into the cerebrospinal fluid. As a result, the presence of desmosterol in the cerebrospinal fluid of patients treated with triparanol has been suggested by these investigators as a means of detecting the presence of brain tumors. The feedback regulation of sterol synthesis in such tumors was not, however, assessed by these authors.

tumor suggests that the method may be applicable to adenocarcinoma and conceivably other tumors as well.

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