[CANCER RESEARCH 38, 4474-4477, December 1978]
0008-5472/78/0038-0000$02.00

Partial "Feedback Control" of β-Hydroxy-β-methylglutaryl Coenzyme A Reductase Activity in Primary Hepatocellular Carcinomas[1]

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ABSTRACT

The activity of β-hydroxy-β-methylglutaryl coenzyme A reductase, the rate-controlling enzyme of cholesterol synthesis, was studied in normal livers and in 64 primary hepatocellular carcinomas from rats fed a basal diet or a diet containing either 2% cholestyramine or 5% cholesterol. The average enzyme activity in hepatocellular carcinomas from rats fed the basal diet was more than twice that in normal liver. Dietary cholesterol caused a reduction in activity to one-ninth of the normal hepatic enzyme activity, whereas cholestyramine feeding resulted in a 7-fold increase above the basal level. The data tended to confirm the previously documented observation that "diet-induced feedback inhibition" of cholesterol synthesis is not expressed in hepatomas, since the enzyme activity was reduced only slightly in cancers from rats fed cholesterol. However, the activities from cancers of cholestyramine-fed rats were 2.7 times greater than those from cholesterol-fed rats. Thus, a degree of control was clearly demonstrable, although it represented only 4% of that seen in normal liver. To our knowledge this is the first report of at least partial "feedback control" of β-hydroxy-β-methylglutaryl coenzyme A reductase activity in hepatocellular carcinomas grown *in vivo*.

INTRODUCTION

In rodents, hepatic cholesterol synthesis is normally finely regulated by the amount of cholesterol that reaches the liver in plasma chylomicrons (34). When cholesterol is added to the diet, the level of chylomicron cholesterol increases, and under normal circumstances, the hepatic synthesis of cholesterol rapidly decreases (33). This effect, referred to as "diet-induced feedback inhibition" is mediated at the level of the microsomal enzyme HMG-CoA reductase.[5] The mechanism of this control is controversial (23), but it is at least partially a result of decreased enzyme synthesis (14). In contrast to normal liver, all hepatocellular carcinomas have been shown to completely lack the diet-induced feedback inhibition of cholesterol synthesis (26–28, 30) and HMG-CoA reductase activity (7, 29).

Feedback regulation of hepatic cholesterol synthesis may also be observed after feeding cholestyramine. The drug is a nonabsorbable anionic exchange resin that binds and depletes the enterohepatic recirculating pool of bile acids (20), secondarily preventing the absorption of cholesterol (21, 31). Even on a cholesterol-free diet, the intestinal lumen normally contains cholesterol derived from bile and from sloughed mucosal cells. Thus, regardless of whether cholesterol alone (34) or cholesterol plus bile acids (11) regulate the activity of HMG-CoA reductase, an increase in the enzyme activity after feeding cholestyramine reflects a release of endogenous feedback inhibition. Since the cholestyramine-mediated stimulation of HMG-CoA reductase is lost in Morris hepatomas (7), it appears that the ability to manifest a difference between diet-induced feedback inhibition and cholestyramine-induced stimulation, referred to as "feedback control," is completely lost in transplantable hepatocellular carcinomas.

A possible explanation for the defective regulation, at least in transplantable hepatomas, is the inability of the carcinomas to take up and accumulate cholesterol from chylomicrons (13). However, one of us (S. Goldfarb) with another group of collaborators (16) recently found that, in a highly differentiated primary hepatocellular carcinoma induced by feeding 2-AAF, the ability to rapidly accumulate dietary cholesterol was not impaired. This suggested that the feedback control of cholesterol synthesis might also be intact in the primary hepatomas. Accordingly, we determined the level of HMG-CoA reductase in 64 primary hepatocellular carcinomas induced by 2-AAF in rats subsequently fed cholestyramine or cholesterol. Our results indicate that the feedback control of HMG-CoA reductase activity, although considerably less than normal, is nevertheless present in primary hepatocellular carcinomas.

MATERIALS AND METHODS

Hepatocellular carcinomas were induced in male Sprague-Dawley rats by *ad libitum* feeding of 2-AAF (m.p. 192–196°) obtained from Aldrich Chemical Co., Inc., Milwaukee, Wis., according to a regimen previously established in this laboratory.[6] The rats were fed 0.04% 2-AAF in ground Mouse Breeder Blox (Allied Mills, Inc., Chicago, Ill.) with added 10% Mazola corn oil. The oil was specifically added to reduce the chance of aerosol dissemination of the carcinogen. After 20 weeks of carcinogen feeding, the rats were fed the same diet, free of carcinogen, for an additional 3 weeks. At the end of that time, groups of rats were fed 1 of 3 different semisynthetic diets, identical to that previously used (7), for 1 week. These contained no additives (basal), 5% cholesterol, or 2% cholestyramine. This level of

[1] This work was supported by National Cancer Institute Grant CA15664.
[2] Recipient of Damon Runyon Postdoctoral Fellowship DRG-60-F.
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[5] The abbreviations used are: HMG-CoA reductase, β-hydroxy-β-methylglutaryl coenzyme A reductase [mevalonate:NADP$^+$ oxidoreductase (coenzyme A acylating) (EC 1.1.1.34); 2-AAF, 2-acetylaminofluorene.
Received October 11, 1977; accepted September 12, 1978.

[6] T. D. Pugh, and S. Goldfarb. Quantitative Histochemical and Autoradiographic Studies of Hepatocarcinogenesis in Rats Fed 2-Acetylaminofluorene followed by Phenobarbital. Cancer Res., in press.

cholestyramine was chosen since it is nontoxic and does not induce steatorrhea (12). During the last feeding period, the rats were cyclically fed according to an established feeding schedule (7) in which the lights were daily turned off at 8 a.m. and on at 8 p.m. and the food was placed in the cages at 8 a.m. and removed at 4 p.m. Sacrifice was at 2 p.m. Only rats that had continued to gain weight and that had large amounts of food in their stomachs at the time of sacrifice were used for the study. Hepatocellular carcinomas and hyperplastic nodules were completely dissected from the surrounding liver tissue, and small sections were taken for histological examination. The remainder of the tissue, freed of any areas of necrosis, was assayed for microsomal HMG-CoA reductase activity (6, 8). Assay conditions were further improved over those previously used by utilizing chromatographically separated β-[^{14}C]hydroxy-β-methylglutaryl CoA as substrate (3). Microsomal protein was determined by the biuret reaction (9). Hepatocellular neoplasms were classified after randomizing all slides and without knowledge of the biochemical data. Carcinomas were distinguished from hyperplastic nodules by evidence of infiltration of surrounding parenchyma and other histological criteria (5, 16, 22). Although hepatocellular carcinomas showed at least focal thickening of hepatic plates and nuclear hyperchromatism, these features were more prominent in the case of the well-differentiated than the highly differentiated hepatocellular carcinomas. The well-differentiated carcinomas also usually showed at least some papillary and glandular formations. Controls for the study consisted of male Sprague-Dawley rats of identical weight, which were fed according to the same regimen as the experimental rats, except that the 2-AAF was deleted from the diet.

RESULTS

A total of 64 hepatocellular carcinomas in 26 rats were available for the study. The carcinomas ranged in size from 100 mg to 4.9 g. However, most carcinomas were rather small, the median size being 450 mg. Fifty of the neoplasms were well-differentiated hepatocellular carcinomas, but only 14 were highly differentiated. Several pure hyperplastic nodules and poorly differentiated carcinomas were also found, but because of their small number, neither group was further evaluated. Nor did we further evaluate the enzymic control of hepatic tissue located between the obvious neoplasms, since hepatocytes in these areas showed varied histology, including some microcarcinomas.

In control animals, HMG-CoA reductase activity was reduced by about 90% after feeding a 5% cholesterol diet (Table 1). In contrast, feeding cholestyramine increased the level of this enzyme activity more than 600% above the level of that in livers of rats fed the basal diet. Thus, there was a 65-fold difference in enzyme activity between the most stimulated and the most inhibited levels. The basal level of enzyme activity of the well-differentiated and highly differentiated hepatomas was more than twice that in the control liver ($p < 0.001$). A significant difference between the mean enzyme activity in hepatocellular carcinomas of rats fed cholesterol or cholestyramine was also apparent, but this was much decreased from that in the control livers. Feeding cholesterol caused a 33% decrease in enzyme activity. Cholestyramine feeding, on the other hand, increased the enzyme activity by 80% in the hepatomas. The differences were considerably more impressive in the small subgroup of highly differentiated hepatocellular carcinomas than in that of well-differentiated hepatomas. The highly differentiated carcinomas from rats fed cholestyramine showed a mean level of HMG-CoA reductase activity that was 4.6 times greater than that from rats fed cholesterol. In contrast the level was only 2.2-fold increased in the case of the well-differentiated hepatomas.

DISCUSSION

Siperstein *et al.* have suggested that the complete loss of dietary-induced feedback inhibition is a general phenomenon that characterizes all hepatocellular carcinomas studied in intact animals (26), including even primary neoplasms (27, 30). Additional studies have even documented this lost control in the nonneoplastic liver from rats (15, 17–19, 25) and trout (24) fed a variety of different carcinogens. We found that cholesterol feeding did cause a small decrease of borderline significance in hepatomas, but even this small change disappeared when the hepatomas were subclassified into highly differentiated and well-differentiated types. Thus, results of our own study tend to support the hypothesis that diet-induced feedback inhibition is not demonstrable in hepatomas.

However, when we compared the average enzyme activity of hepatomas from rats fed cholesterol with that from hepatomas of rats fed cholestyramine, we did find highly significant differences. Furthermore, the range of feedback control in the highly differentiated carcinomas was more than twice that in the well-differentiated hepatomas. This is of particular interest since highly differentiated carcinomas also more rapidly accumulate dietary cholesterol than do well-differentiated or poorly differentiated neoplasms (16). The findings therefore suggest that the degree of deranged cholesterol uptake and feedback control in general correlate with histological evidence of tumor progression. Clearly, however, this conclusion, based on our study of 64 neoplasms, does not refute the well-established principle that, during tumor progression in specific neoplasms, individual characteristics progress independently of each other (4).

Originally, it was hypothesized that the derangement in regulation of cholesterol synthesis in hepatomas reflected an abnormality of enzyme structure (27), but this was subsequently proven not to be the case (1). The alteration probably reflects an inability to accumulate and retain cholesterol carried by chylomicrons. This might be due to an alteration of blood supply (10), a derangement at the level of the cell membrane (analogous to the decrease in receptors for low-density lipoproteins in fibroblasts of patients with type II hyperlipoproteinemia) (2), or a combination of the 2 effects. We speculate that a diminished and predominantly arterial blood supply depleted of some factor normally present in portal venous blood is probably quite important. This concept is based upon the observation that transplantable hepatomas, which receive only arterial

Table 1

HMG-CoA reductase activity in control livers and HC[a] of rats fed different diets

	HMG-CoA reductase activity (nmol mevalonate/mg microsomal protein/hr)					
	Basal diet (9)		Cholesterol diet (10)		Cholestyramine diet (7)	
Control livers	16.6 ± 3.1[b]	(7)[c]	1.8 ± 0.4[d]	(8)	112.6 ± 16.0[d]	(7)
Highly differentiated HC	41.2 ± 10.7	(3)	17.3 ± 4.3[e, f]	(3)	79.4 ± 14.1	(8)
Well-differentiated HC	37.5 ± 5.1	(15)	26.5 ± 2.8[e, g]	(24)	59.5 ± 8.8[h]	(11)
Well-differentiated and highly differentiated HC	38.1 ± 4.6	(18)	25.5 ± 2.6[i]	(27)	67.8 ± 8.1[j]	(19)

[a] HC, hepatocellular carcinomas.
[b] Mean ± S.E.
[c] Numbers in parentheses, number of rats sacrificed or number of HC.
[d] Difference from basal diet-fed rats: $p < 0.001$.
[e] Difference from highly differentiated or well-differentiated HC of basal diet-fed rats is not significant.
[f] Difference from highly differentiated HC of cholestyramine diet-fed rats: $p < 0.005$.
[g] Difference from well-differentiated HC of cholestyramine diet-fed rats: $p < 0.005$.
[h] Difference from well-differentiated HC of basal diet-fed rats: $p < 0.05$.
[i] Difference from well-differentiated and highly differentiated HC of basal diet-fed rats: $p < 0.025$. Difference from well-differentiated and highly differentiated HC of cholestyramine diet-fed rats: $p < 0.001$.
[j] Difference from well-differentiated and highly differentiated HC of basal diet-fed rats: $p < 0.005$.

blood, show a complete loss of feedback control and inability to take up and retain cholesterol, whereas primary hepatomas, some of which have a portal blood supply (32), are capable of accumulating chylomicron cholesterol and do manifest a certain degree of feedback control.

REFERENCES

1. Brown, M. S., Dana, S. E., and Siperstein, M. D. Properties of 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase Solubilized from Rat Liver and Hepatoma. J. Biol. Chem., *249:* 6585–6589, 1974.
2. Brown, M. S., Goldstein, J. L., and Siperstein, M. D. Regulation of Cholesterol Synthesis in Normal and Malignant Tissue. Federation Proc., *32:* 2168–2173, 1973.
3. Fogelman, A. M., Edmond, J., Seager, J., and Popják, G. Abnormal Induction of 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase in Leukocytes from Subjects with Heterozygous Familial Hypercholesterolemia. J. Biol. Chem., *250:* 2045–2055, 1975.
4. Foulds, L. Multiple Etiologic Factors in Neoplastic Development. Cancer Res., *25:* 1339–1347, 1965.
5. Goldfarb, S. A Morphological and Histochemical Study of Carcinogenesis of the Liver in Rats Fed 3′-Methyl-4-dimethylaminoazobenzene. Cancer Res., *33:* 1119–1128, 1973.
6. Goldfarb, S., and Pitot, H. C. An Improved Assay of β-Hydroxy-β-methylglutaryl Coenzyme A Reductase. J. Lipid Res., *12:* 512–515, 1971.
7. Goldfarb, S., and Pitot, H. C. The Regulation of β-Hydroxy-β-methylglutaryl Coenzyme A Reductase in Morris Hepatomas 5123C, 7800, and 9618A. Cancer Res., *31:* 1879–1882, 1971.
8. Goldfarb, S., and Pitot, H. C. Stimulatory Effect of Dietary Lipid and Cholestyramine on Hepatic HMG CoA Reductase. J. Lipid Res., *13:* 797–801, 1972.
9. Gornall, A. G., Bardawill, C. J., and David, M. M. Determination of Serum Proteins by Means of the Biuret Reaction. J. Biol. Chem., *177:* 751–766, 1949.
10. Gullino, P. M., and Grantham, F. H. Studies on the Exchange of Fluids between Host and Tumor. II. The Blood Supply of Hepatomas and Other Tumors in Rats and Mice. J. Natl. Cancer Inst., *27:* 1465–1491, 1961.
11. Hamprecht, B., Roscher, R., Waltinger, G., and Nüssler, C. Influence of Bile Acids on the Activity of Rat Liver 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase. 2. Effect of Cholic Acid in Lymph Fistula Rats. European J. Biochem., *18:* 15–19, 1971.
12. Harkins, R. W., Hagerman, L. M., and Sarett, H. P. Absorption of Dietary Fats by the Rat in Cholestyramine-induced Steatorrhea. J. Nutr. *87:* 85–92, 1965.
13. Harry, D. S., Morris, H. P., and McIntyre, N. Cholesterol Biosynthesis in Transplantable Hepatomas: Evidence for Impairment of Uptake and Storage of Dietary Cholesterol. J. Lipid Res., *12:* 313–317, 1971.
14. Higgins, M., and Rudney, H. Regulation of Rat Liver β-Hydroxy-β-methylglutaryl CoA Reductase Activity by Cholesterol. Nature New Biol., *246:* 60–61, 1973.
15. Horton, B. J., Horton, J. D., and Sabine, J. R. Metabolic Controls in Precancerous Liver. II. Loss of Feedback Control of Cholesterol Synthesis, Measured Repeatedly *in Vivo*, during Treatment with the Carcinogens *N*-2-Fluorenylacetamide and Aflatoxin. European J. Cancer, *8:* 437–443, 1972.
16. Horton, B. J., Mott, G. E., Pitot, H. C., and Goldfarb, S. Rapid Uptake of Dietary Cholesterol by Hyperplastic Liver Nodules and Primary Hepatomas. Cancer Res., *33:* 460–464, 1973.
17. Horton, B. J., and Sabine, J. R. Metabolic Controls in Precancerous Liver: Defective Control of Cholesterol Synthesis in Rats Fed *N*-2-Fluorenylacetamide. European J. Cancer, *7:* 459–465, 1971.
18. Horton, B. J., and Sabine, J. R. Metabolic Controls in Precancerous Liver. III. Further Studies on the Control of Lipid Synthesis during *N*-2-Fluorenylacetamide Feeding. European J. Cancer, *9:* 1–9, 1973.
19. Horton, B. J., and Sabine, J. R. Metabolic Controls in Precancerous Liver. IV. Loss of Feedback Control of Cholesterol Synthesis and Impaired Cholesterol Uptake in Ethionine-fed Rats. European J. Cancer, *9:* 11–17, 1973.
20. Huff, J. W., Gillfillan, J. L., and Hunt, V. M. Effect of Cholestyramine, a Bile Acid Binding Polymer, on Plasma Cholesterol and Fecal Bile Acid Excretion in the Rat. Proc. Soc. Exptl. Biol. Med., *114:* 352–355, 1963.
21. Hyun, S. A., Vahouny, G. V., and Treadwell, C. R. Effect of Hypocholesterolemic Agents on Intestinal Cholesterol Absorption. Proc. Soc. Exptl. Biol. Med., *112:* 496–501, 1963.
22. Reuber, M. D. Histopathology of Transplantable Hepatic Carcinomas Induced by Chemical Carcinogens in Rats. Gann Monograph, *1:* 43–54, 1966.
23. Rodwell, V. W., Nordstrom, J. L., and Mitschelen, J. J. Regulation of HMG CoA Reductase. Advan. Lipid Res., *14:* 1–74, 1976.
24. Siperstein, M. D. Feedback Control of Cholesterol Synthesis in Normal, Malignant and Premalignant Tissues. Proc. Can. Cancer Res. Conf., *7:* 152–162, 1966.
25. Siperstein, M. D. Deletion of the Cholesterol Negative Feedback System in Precancerous Liver. J. Clin. Invest., *45:* 1073, 1966.
26. Siperstein, M. D. Regulation of Cholesterol Biosynthesis in Normal and Malignant Tissues. Current Topics Cellular Regulation, *2:* 65–100, 1970.
27. Siperstein, M. D., and Fagan, V. M. Deletion of the Cholesterol-negative Feedback System in Liver Tumors. Cancer Res., *24:* 1108–1115, 1964.
28. Siperstein, M. D., Fagan, V. M., and Morris, H. P. Further Studies on the Deletion of the Cholesterol Feedback System in Hepatomas. Cancer Res., *26:* 7–11, 1966.
29. Siperstein, M. D., Gyde, A. M., and Morris, H. P. Loss of Feedback Control of Hydroxymethyl Coenzyme A Reductase in Hepatomas. Proc.

Natl. Acad. Sci. U. S., *68:* 315–317, 1971.
30. Siperstein, M. D., and Luby, L. J. Control of Cholesterol Synthesis in Normal and Malignant Tissue. *In:* O. W. Neuhass and J. E. Halver (eds.), Fish in Research, pp. 87–102. New York: Academic Press, Inc., 1969.
31. Siperstein, M. D., Nichols, C. W., and Chaikoff, I. L. Effects of Ferric Chloride and Bile on Plasma Cholesterol and Atherosclerosis in the Cholesterol Fed Bird. Science, *117:* 386–389, 1953.
32. Solt, D. B., Hay, J. B., and Farber, E. Comparison of the Blood Supply of Dimethylnitrosamine-induced Hyperplastic Nodules and Hepatomas and to the Surrounding Liver. Cancer Res., *37:* 1686–1691, 1977.
33. Taylor, C. B., and Gould, R. G. Effect of Dietary Cholesterol on Rate of Cholesterol Synthesis in the Intact Animal Measured by Means of Radioactive Carbon. Circulation, *2:* 467–468, 1950.
34. Weis, H. J., and Dietschy, J. M. Failure of Bile Acids to Control Hepatic Cholesterogenesis: Evidence for Endogenous Cholesterol Feedback. J. Clin. Invest., *48:* 2398–2408, 1969.