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Rapid Uptake of Dietary Cholesterol by Hyperplastic Liver Nodules and Primary Hepatomas

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SUMMARY

Hyperplastic liver nodules and primary hepatomas induced by 2-acetylaminofluorene take up cholesterol-^{3}H, given p.o., at a rate only slightly less than that in normal liver, and 10- to 30-fold greater than that reported for transplantable hepatomas. In the case of primary hepatomas, the rate of uptake appeared to be related to the degree of differentiation and hence a function of tumor progression. Previous studies on regulation of cholesterol synthesis in primary hepatomas and cholesterol uptake studies in transplantable hepatomas may need to be reinterpreted in view of these results.

INTRODUCTION

The loss of inhibition of cholesterol synthesis in response to cholesterol feeding is reported to be a consistent feature in both primary and transplantable hepatomas (17). Harry *et al.* (6) showed that dietary cholesterol-^{14}C accumulates much more slowly in transplantable hepatomas than in the host livers and suggested that this, rather than a specific intracellular defect, is the cause of the lack of response to cholesterol feeding. [This is a reasonable suggestion since it is known that dietary cholesterol does appear to regulate the level of HMG-CoA reductase[4] (11), the rate-limiting enzyme of cholesterol synthesis, although the mechanism of regulation is not clear.]

In this study the rate of cholesterol-^{3}H uptake in hyperplastic liver nodules and into primary hepatomas was measured, since both have been reported to have defective control of cholesterol synthesis, similar to that found in transplantable hepatomas (7, 17).

MATERIALS AND METHODS

Animals and Diets. Male, Sprague-Dawley CFE rats (Carworth Farms, Inc., New City, N. Y.), which weighed 100 g at the beginning of the regimen, were fed *ad libitum* a powdered commercial laboratory ration (Wayne Lab Blox, Allied Mills, Inc., Chicago, Ill.) with 0.06% AAF (Mann Research Laborabories, Inc., New York, N. Y.) for 3 weeks, followed by 1 week of the chow diet alone. This feeding cycle was repeated for a total of 4 times after which the rats were maintained on the chow alone. This regimen has been reported to induce a high incidence of hyperplastic liver nodules and hepatocellular carcinomas (19). Hyperplastic liver nodules were studied in these rats 18 to 20 weeks after commencement of AAF feeding, and the primary hepatomas were studied 32 weeks after feeding. All rats were subjected to a 12-hr-day:12-hr-night regimen (lights on at 8:00 p.m. and off at 8:00 a.m.).

Measurement of Cholesterol-^{3}H Uptake. Rats were dosed p.o. with 5 μCi cholesterol-7-^{3}H per kg (New England Nuclear, Boston, Mass.) in 0.5 ml olive oil, at 7:30 a.m. (*i.e.*, at the time when the rats normally began eating) and killed 4 hr later. This time period was selected because it has been shown that only a small fraction of the chylomicron cholesterol taken up by the liver is released into the plasma again within 4 hr (12). Samples (20- to 100-mg) of liver, hepatomas, and nodules and 0.5 ml serum were saponified in 0.5 ml 10% KOH (70°/2 hr) and extracted once in 10.0 ml hexane after the addition of 0.5 ml ethanol. Aliquots were evaporated to dryness to measure total cholesterol [by gas-liquid chromatography, (14)] and cholesterol-^{3}H in 10 ml Scintisol Complete in a Packard Tri-Carb Model 3375 spectrometer.

Histological Analysis. Paraffin sections prepared from each hepatoma, from representative nodules, and from surrounding liver were stained with hematoxylin and eosin.

RESULTS

Hyperplastic Liver Nodules. Well-delineated, tan hepatocellular nodules (4 to 10 mm in diameter, 20 to 90 mg) were easily dissected out. They were composed of large hydropic-appearing hepatocytes (Fig. 1) typical of the type in hyperplastic nodules associated with hepatoma development (2, 4). The hepatocytes in the surrounding liver showed some cytological variation but in general had the appearance of those in normal liver, although many small clusters were surrounded by septa containing proliferating ductules. The uptake of cholesterol-^{3}H 4 hr after dosing is shown in Table 1.

In 3 of the 4 nodular livers there was a significant ($p < 0.01$) difference between cholesterol uptake into the nodules and into normal liver tissue in the same animal. However, the

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[4] The abbreviations used are: HMG-CoA reductase, β-hydroxy-β-methylglutaryl coenzyme A reductase, AAF, 2-acetylaminofluorene (*N*-2-fluorenylacetamide).

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actual degree of difference is generally not great, and the uptake is 20- to 30-fold greater than that reported in transplantable hepatomas in the same time (6). Since the level of cholesterol in the nodular tissue was never more than 10% lower than in the surrounding liver, it is unlikely that differences in the size of the cholesterol pool affected the rate of cholesterol-^{3}H uptake.

It was of particular interest that the ratio of cholesterol-^{3}H uptake into nodules compared to the surrounding liver showed marked differences between animals, but within each rat the individual nodules appeared to form a remarkably homogeneous population.

Primary Hepatomas. Seven tumors, all hepatomas, measuring 1.5 to 5 cm in diameter were found in 3 livers (Table 2). The hepatomas–soft, brownish tan and free of necrosis–were dissected free of surrounding parenchyma. Portions taken for histology were classified according to the criteria of Reuber (15) into poorly, well-, or highly differentiated hepatomas (Table 2; Figs. 2 to 4). Except for occasional areas of residual hyperplastic nodules and some mild fibrosis and cyst formation, the surrounding parenchyma was unremarkable. All 3 rats that had hepatocellular carcinomas also had characteristic early infiltrating squamous cell carcinomas of the ear duct gland typical of the type described in rats fed AAF (10).

Portions of hepatoma and surrounding liver were taken for cholesterol assay and for assay of cholesterol-^{3}H. The results in Table 2 indicate that there was a significant impairment of cholesterol-^{3}H uptake in all of the primary hepatomas, but again the actual rate of uptake was much higher than reported for transplantable hepatomas. In addition, the cholesterol level in the hepatomas was 10 to 25% higher than in the surrounding liver. Therefore, although the specific activity of cholesterol in all the hepatomas was less than in the liver, the actual uptake of cholesterol-^{3}H per g of tissue was not significantly different than in the liver for 2 of the most highly

Table 1

Uptake of cholesterol-^{3}H by hyperplastic liver nodules

Samples of serum, nodules, and "normal" liver from each animal were taken 4 hr after treatment with cholesterol-^{3}H.

Experiment	Rat	Tissue	n^a	mg cholesterol/g tissue	cpm × 10^{-3}/g tissue	cpm × 10^{-3}/mg cholesterol	S.A.[b] tissue/S.A. serum
1	1	Nodules	16	2.00 ± 0.03[c,d]	12.1 ± 0.4[e]	6.07 ± 0.19[e]	0.54 ± 0.02[e]
		Liver	8	2.30 ± 0.09	20.7 ± 0.5	9.09 ± 0.41	0.81 ± 0.04
	2	Control liver	5	1.83 ± 0.06	15.0 ± 0.2	7.60 ± 0.44	0.61 ± 0.04
	3	Control liver	5	1.74 ± 0.05	5.4 ± 0.2	3.09 ± 0.09	0.56 ± 0.01
2	1	Nodules	5	2.41 ± 0.05[f]	52.8 ± 2.3[e]	21.86 ± 0.77[e]	0.43 ± 0.01[e]
		Liver	5	2.58 ± 0.03	102.9 ± 3.3	39.96 ± 1.54	0.79 ± 0.03
	2	Nodules	6	2.64 ± 0.03[d]	23.9 ± 0.7	8.90 ± 0.15	0.70 ± 0.01
		Liver	6	2.80 ± 0.03	25.8 ± 1.0	9.19 ± 0.30	0.72 ± 0.02
	3	Nodules	8	2.67 ± 0.06	35.1 ± 1.0[f]	13.02 ± 0.52[d]	0.54 ± 0.02[d]
		Liver	8	2.67 ± 0.09	47.2 ± 4.3	17.47 ± 1.05	0.72 ± 0.04

[a] *n*, number of nodules or liver samples taken from each animal.
[b] S.A., specific activity of cholesterol-^{3}H.
[c] Mean ± S.E.
[d,e,f] Significantly different from "normal" liver from the same animal; $p < 0.01$, $p < 0.001$, $p < 0.05$, respectively.

Table 2

Uptake of cholesterol-^{3}H by primary hepatomas

Samples of serum, primary hepatomas and "normal" liver from each animal were taken 4 hr after treatment with cholesterol-^{3}H, as in Table 1.

Rat	Tissue	n^a	mg cholesterol/g tissue	cpm × 10^{-3}/g tissue	cpm × 10^{-3}/mg cholesterol	S.A.[b] tissue/S.A. serum	Degree of differentiation
1	Hepatoma A	3	3.10 ± 0.21[c,d]	17.4 ± 3.3[d]	5.79 ± 1.36[e]	0.39 ± 0.09[e]	Well
	Hepatoma B	3	2.67 ± 0.23	23.9 ± 1.7	9.04 ± 0.88[d]	0.61 ± 0.06[d]	Highly
	Hepatoma C	3	2.95 ± 0.17[d]	22.1 ± 0.7[f]	7.57 ± 0.64[e]	0.51 ± 0.04[f]	Well
	Hepatoma D	3	3.05 ± 0.03[d]	14.9 ± 0.4[f]	4.89 ± 0.18[f]	0.33 ± 0.01[f]	Well
	Liver	6	2.39 ± 0.11	28.0 ± 0.8	11.78 ± 0.45	0.80 ± 0.03	
2	Hepatoma	4	3.08 ± 0.09[f]	39.0 ± 1.1[f]	12.74 ± 0.75[f]	0.34 ± 0.02[f]	Well
	Liver	4	2.20 ± 0.06	74.0 ± 4.1	33.48 ± 1.16	0.90 ± 0.03	
3	Hepatoma A	3	3.03 ± 0.27[d]	6.0 ± 0.9[f]	2.07 ± 0.51[f]	0.09 ± 0.02[f]	Poorly
	Hepatoma B	3	3.05 ± 0.11[e]	30.1 ± 3.5	9.80 ± 0.80[e]	0.43 ± 0.03[e]	Well
	Liver	4	2.20 ± 0.14	33.0 ± 3.3	14.91 ± 0.70	0.66 ± 0.03	

[a] *n*, number of nodules or liver samples taken from each animal.
[b] S.A., specific activity of cholesterol-^{3}H.
[c] Mean ± S.E.
[d,e,f] Significantly different from "normal" liver from the same animal; $p < 0.05$, $p < 0.01$, $p < 0.001$, respectively.

differentiated hepatomas.

The histological analysis indicated a good correlation between the degree of differentiation and rate of cholesterol-^{3}H uptake in each hepatoma.

DISCUSSION

The studies presented here have shown that cholesterol uptake is significantly slower in both hyperplastic liver nodules and primary hepatomas than it is in normal liver, but it is still 10 to 30 times faster than the rate previously reported for transplantable hepatomas (6). This small difference in uptake does not appear to be sufficient to explain the complete lack of control of cholesterol synthesis which has been reported for primary hepatomas. Furthermore, the rate of uptake in primary hepatomas appeared to be closely related to the degree of differentiation. The loss of inhibition of cholesterol synthesis has been reported in only 2 human hepatomas (17) and in 1 primary trout hepatoma, while studies on primary mouse hepatomas gave equivocal results (9).

Harry *et al.* (6) reported that transplantable hepatomas, in contrast to host livers, do not rapidly accumulate cholesterol after a single dose. This suggested that the defective inhibition of cholesterol synthesis might be due to an abnormality of membrane transport and uptake of chylomicron cholesterol in neoplastic cells. However, in view of our findings that primary hepatomas do not show a similar decrease in uptake, 3 possibilities must be seriously considered.

First, the defect in cholesterol uptake in transplantable hepatomas may not be a consequence of the malignant change at all. It could be due to the decreased or abnormal blood supply. This is suggested since transplantable hepatomas have an arterial blood supply while normal liver and probably primary hepatomas (1) derive up to 75% of their blood from the portal vein. In addition, some transplantable hepatomas have been found to have only 5% of the blood supply of the host liver (5). Thus a blood-borne substance would traverse the liver 20 times for each passage through the hepatoma. Such a difference may be extremely important in the case of chylomicron cholesterol uptake, which is cleared extremely rapidly by the normal liver (13).

Secondly, since primary, well-differentiated hepatomas do accumulate dietary cholesterol, inhibition of cholesterol synthesis in response to cholesterol feeding may occur in a fashion similar to normal liver. This possibility is supported by the finding of Watson (20) that cholesterol synthesis in hepatomas in cell culture is inhibited in response to cholesterol in the culture medium. However, this regulation found in tissue culture is not necessarily analogous to inhibition of cholesterol synthesis by dietary cholesterol in the whole animal. We are now attempting to produce sufficiently large numbers of well-differentiated primary hepatomas to examine this hypothesis.

Thirdly, if well-differentiated primary hepatomas do in fact lack inhibition of cholesterol synthesis, it is likely to be a result of an intracellular defect rather than a defect of cell membrane transport as suggested by the earlier results (6). In this respect, primary hepatomas may resemble precancerous liver more closely than transplantable hepatomas. Livers of animals treated with ethionine (8) or AAF (B. J. Horton, J. Horton and H. C. Pitot, manuscript in preparation) show a loss of inhibition of cholesterol synthesis, but take up cholesterol-^{3}H at a rate similar to those of the nodules and primary hepatomas examined here.

Our earlier studies (3) and those of Siperstein *et al.* (18) indicated that the lack of inhibition of cholesterol synthesis in transplantable hepatomas reflects the unresponsiveness of the rate-controlling enzyme in cholesterol synthesis, HMG-CoA reductase. Therefore the defect may be in the regulation of the amount of this enzyme. If so, this must be a particularly specific regulatory defect, since it has been shown that the diurnal rhythm of HMG-CoA reductase (3) and cholesterol synthesis (16) is retained in some hepatomas that lack inhibition of cholesterol synthesis in response to cholesterol feeding.

These results emphasize the danger of relying only on results obtained from transplantable tumors, which may be unrelated to the situation in primary tumors simply due to such factors as alterations in the blood supply.

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Figs. 1 to 4. All sections are stained with H & E, × 200.

Fig. 1. A typical hyperplastic nodule. The hepatocytes have abundant foamy cytoplasm and large nuclei. Surrounding hepatocytes are visible in *upper right.*

Fig. 2. Highly differentiated hepatocellular carcinoma (Table 2, Rat 1B). The carcinoma cells are arranged in 1-cell-thick plates and contain abundant cytoplasm.

Fig. 3. Well-differentiated hepatocellular carcinoma (Table 2, Rat 1D). The carcinoma cells are arranged in 2- to 5-cell-thick plates. The nuclei are generally uniform and mitoses are rare.

Fig. 4. Poorly differentiated hepatocellular carcinoma (Table 2, Rat 3A). The trabecular pattern is still apparent, but there is marked anaplasia and frequent mitoses.