The Feedback Control of Hepatic Cholesterol Synthesis in Ugandan Patients with Liver Disease

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SUMMARY

Hepatic cholesterol synthesis is thought to be under the influence of a feedback inhibition by dietary cholesterol, except in the case of primary hepatoma. This feedback mechanism has been studied in Ugandan patients with hepatoma and with nonneoplastic liver disease. A control study of a group of patients in Boston was also carried out.

The functioning of the feedback mechanism in Ugandans with hepatoma could not be analyzed in that all hepatoma biopsies synthesized very low amounts of cholesterol. Cholesterol synthesis by hepatoma tissue was significantly less than that of Ugandan controls and was also less than that of previously studied hepatomas in the United States.

In patients with nonneoplastic liver disease, the feedback mechanism in several cases appeared to be absent. This finding was in contrast to results from the group of control patients in Boston, which findings verified the cholesterol feedback phenomenon previously reported in Westerners. The Ugandan patients lacking the feedback mechanism did not appear to fall into any single clinical or histological category. The reasons for this finding are uncertain, although a possible role of aflatoxin is suggested.

INTRODUCTION

Hepatic cholesterol synthesis appears to be under the influence of a negative feedback by dietary cholesterol, as documented in dogs and rats (8) and also in man (5). The feedback mechanism, moreover, remains demonstrable in the presence of various liver diseases (5), with the exception of primary hepatoma. In rat hepatomas and in 2 human hepatomas, absence of the feedback mechanism has been found (19, 20), and it has been suggested that this condition is unique, among diseases of the liver, to primary hepatoma.

In Uganda, primary hepatoma is 20-fold more common than in North America (10). Although it has been suggested that hepatitis and cirrhosis precede the development of a hepatoma (15, 22), the etiology of the disease in Ugandans remains obscure. The present study of Ugandan hepatomas was undertaken to investigate the reported absence of the cholesterol feedback mechanism in hepatoma in a larger group of patients than is generally available in this country and to study patients with nonneoplastic liver disease. The possibility was considered that absence of the cholesterol feedback mechanism might appear in a histologically or clinically definable group of patients without evidence of hepatoma.

A control study of patients in Boston was carried out to verify the presence of the cholesterol feedback mechanism in Westerners that was previously reported (5). The Ugandans with hepatoma described here form part of an earlier report (2).

MATERIALS AND METHODS

Assay of Hepatic Cholesterol Synthesis. The in vitro method of Bhattathiry and Siperstein (5) was used. Needle biopsy pieces of liver (2 to 22 mg, wet weight) were incubated for 2 hr with 2 μCi sodium acetate-2-14C; specific activity, 2 μCi/μmole (New England Nuclear, Boston, Mass.). Control assays were done with rat liver samples cut freehand to similar sizes and shapes and handled identically. Digitonin-precipitable sterol-14C and 14CO2 were quantitated by liquid scintillation counting. Of the added radioactivity, 2 to 10% was recovered as 14CO2. We controlled the extraction procedures by carrying cholesterol-14C (New England Nuclear) through some or all of the postincubation steps. The average recovery in the digitonide precipitate was 95%. From the standard incubation, the portion of the radioactive digitonide that represented cholesterol was estimated with regeneration of the free sterols (21) and chromatography on Silica Gel G against a cholesterol standard with benzene:ethyl acetate (5:1). An average 83% of the radioactivity migrated with cholesterol, a result similar to that obtained by Dietsch and Siperstein (7).

Animal Studies. Sprague-Dawley rats, 150 to 250 g, were given a synthetic cholesterol-free diet (Nutritional Biochemicals Corp., Cleveland, Ohio) with 5% added safflower oil. The same formulation with 0.5% added cholesterol was a high-cholesterol diet. The data of Table 1 confirm the known effect of dietary cholesterol on hepatic cholesterol synthesis in the rat. The average reproducibility of the assay was found to
be 14% (range, 4 to 34%), a variability similar in magnitude to that reported by others (7, 13, 23).

Human Studies. Liver tissue from needle biopsies was dropped immediately into cold Krebs-Ringers bicarbonate buffer (pH 7.4) and was maintained at 4°C until the incubation was carried out, which was always within 1 hr. Hepatoma tissue from 2 patients was obtained at surgery. The assay for endogenous cholesterol synthesis was carried out on a portion of the tissue directly adjacent to that sent for histology. After incubation, extraction of $^{14}$CO$_2$, and addition of potassium hydroxide to 40% final concentration, the samples were transported back to Boston for the isolation and measurement of cholesterol-1$^{14}$C (5). Interim storage was at 0—4°C, for periods up to 4 months. In control experiments, the handling of rat liver in the same manner produced no significant change in digitonin-precipitable radioactivity. All patients studied were well enough to eat; all were on a hospital diet for at least 4 days prior to biopsy. The "house" diet at Mulago Hospital was free of eggs and virtually all milk products. This diet has 4 g cholesterol daily in the form of extra eggs with each meal.

The result in a small group of patients hospitalized in Boston qualitatively confirmed the data reported previously (5) (Chart 1). Diagnoses from the Boston patients included: hepatitis (toxic and viral), portal cirrhosis, fatty metamorphosis, drug-induced cholestasis, secondary carcinoma, sarcoid, tuberculosis, and normal liver.

Deionized water was used for all media and for washing incubation glassware. For avoidance of variations in assay conditions between Boston and Uganda, the necessary reagents and glassware were shipped to Uganda from Boston.

RESULTS

Chart 1 presents, by diagnosis, hepatic cholesterol synthesis in a group of patients after at least 3 days of either a house diet or a high-cholesterol diet. The group designated "normal" were patients biopsied because of clinical suspicion of liver pathology but whose liver proved to be histologically normal. The group designated "other" were patients whose primary disease was extrahepatic and whose manifestations of liver disease were minor. Diagnoses for this group included bacterial pneumonia, amebiasis, and tuberculosis. Of particular interest are the patients with hepatoma (Chart 1), whose tissue synthesized barely measurable amounts of cholesterol regardless of previous diet. The respective hepatoma specimens were graded for cellular differentiation (kindly done by Professor M. S. R. Hutt, Makerere Medical College, Kampala, Uganda), and all grades were represented. Tissue from patients

![Graph showing incorporation of acetate into cholesterol](chart.png)

**Table 1**

<table>
<thead>
<tr>
<th>Cholesterol diet</th>
<th>$^{14}$CO$_2$</th>
<th>Cholesterol-1$^{14}$C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (8)</td>
<td>1765 ± 297</td>
<td>606 ± 267</td>
</tr>
<tr>
<td>High (8)</td>
<td>2278 ± 608</td>
<td>20 ± 16</td>
</tr>
</tbody>
</table>

* a No. in parentheses, no. of animals.
* b Mean synthesis ± S.D.; units as in Table 1, Footnote b.
* c $p < 0.001.$
with hepatitis (Chart 1) also synthesized less than that of a control group. The findings are summarized in Table 2.

Chart 1 also demonstrates that, as a group, the values from Ugandans on either diet varied widely, with much overlap. There is no significant difference in cholesterol synthesis between the combined house and high-cholesterol groups, in contrast to Boston patients, who show a distinct inhibition of cholesterol synthesis on a high-cholesterol diet.

In 8 Ugandan patients, biopsies were obtained both before and after a high-cholesterol diet. The paired data confirms the variable response to a high-cholesterol diet in Ugandans (Table 3).

DISCUSSION

The study produced 2 unexpected findings. The level of synthesis in hepatoma tissue was so low that the presence or absence of feedback inhibition by dietary cholesterol could not be assessed. Previous reports on 2 hepatomas in the United States (19, 20) indicated that 76 to 94 mmoles of acetate were incorporated into cholesterol per 2 hr of incubation. A single hepatoma assayed in Boston produced similar values (65 and 91 mmoles, on duplicate assay). The low levels of cholesterol synthesis by Ugandan hepatomas may reflect a greater biochemical dedifferentiation than that which exists in the African tumors. African hepatomas apparently synthesize α-fetoprotein more frequently than do hepatomas in the United States (1), a finding that suggests a greater degree of embryonal dedifferentiation in the African tumors. However, hepatitis specimens also synthesized significantly less cholesterol than did controls; this suggests that inflammation and necrosis also may affect the level of hepatic cholesterol synthesis.

In contrast to the group of Boston patients, an absence of the cholesterol feedback mechanism was noted in Ugandans who had no evidence of hepatoma, clinically or histologically. In these patients, absence of the feedback mechanism did not correlate with histological diagnosis, tribe, nutritional state, or biochemical data. There was no significant hypercholesterolemia in any patient, if indeed this factor plays a role in the level of hepatic cholesterol synthesis in man (12, 16). The actual consumption of the egg diet was carefully monitored to be certain of the intake. A physiological barrier to cholesterol absorption was not ruled out. However, recent studies on the Masai tribe of Kenya show that their intestinal cholesterol absorption rates actually exceed those of the average Westerner (6). A subclinical malabsorptive disorder was not specifically excluded in any patient. Apart from about one-half of the hepatoma patients, however, none of the patients studied had histories of malnourishment, were grossly underweight by African standards, or had diarrhea. Moreover, sprue is apparently rare in Africa (11).

Hereditary or acquired differences in the body-cholesterol economy of Ugandans and Westerners may eventually become apparent and provide an explanation for the observed differences in their respective responses to a high-cholesterol diet. The possibility that aflatoxin plays a role deserves mention, however. The high incidence of hepatoma in Africa has been the object of many studies, and various etiological hypotheses have been suggested (15, 22), including more recently the fungal toxin aflatoxin (3). Experimentally, aflatoxin is an extremely potent hepatocarcinogen. It has been found in significant quantity in domestic Ugandan foodstuffs (3, 14), and its presence correlated with hepatoma incidence (4). Therefore, aflatoxin may be the cause of at least some of the liver disease of Ugandans.

Relevant to the present study is experimental work showing an effect of aflatoxin on the feedback control of hepatic cholesterol synthesis. The feeding of aflatoxin to rats or trout apparently causes deletion of the cholesterol feedback mechanism in the liver. The effect is seen, moreover, weeks before the development of a primary hepatoma (18).

The present study reveals only a lack of feedback inhibition in a number of patients and does not relate this finding to either hepatoma or aflatoxin ingestion. Nevertheless, the possibility remains that such findings represent an aflatoxin effect in these patients and, possibly, a "precancerous" biochemical lesion.

ACKNOWLEDGMENTS

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