# AUTONOMOUS CHOLESTEROL AND FATTY ACID SYNTHESIS IN HEPATOMAS: DELETION OF THE ADENOSINE 3', 5'-CYCLIC MONOPHOSPHATE CONTROL MECHANISM OF NORMAL LIVER

Lee A. Bricker and Gerald S. Levey

Divisions of General Medicine and Endocrinology and Metabolism
Department of Medicine, University of Miami School of Medicine
Miami, Florida 33152

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Summary: Rat hepatoma slices incubated with cAMP ($5x10^{-3}M$) or dibutyryl cAMP ($3x10^{-4}M$), and acetate-2-$^{14}C$, demonstrated unsuppressible cholesterol and fatty acid synthesis, compared to controls. This observation contrasts sharply with that of the marked suppression in lipogenesis brought about by cyclic nucleotides in normal liver and indicates a deletion in hepatomas of this regulatory mechanism in lipogenesis.

We have previously reported that cholesterol and fatty acid synthesis in normal liver is suppressible by adenosine 3', 5'-cyclic monophosphate (cAMP) (1) and have suggested the possibility that this is a physiologically meaningful mechanism in the regulation of hepatic lipogenesis. The sequence of events in liver appears to be quite specific, apparently involving an early step in the utilization of acetyl CoA. Similar considerations involving hepatomas presented themselves, since many of these malignant tumors likewise are highly active producers of sterols (2). Specific defects in regulation of tumor sterologenesis have been well described previously (3,4), involving, primarily, the conversion of β-hydroxy-β-methylglutaryl CoA to mevalonate. The purpose of this investigation was to examine the possibility that a separate regulatory defect in lipogenesis related to cAMP exists in these neoplasms.

## MATERIALS AND METHODS

The experimental protocol has been described previously (1). The procedure utilized tissue slices incubated in Krebs' bicarbonate buffer with 2 μCi acetate-2-$^{14}C$, and either a final concentration of cAMP of $5x10^{-3}M$, or of dibutyryl cAMP, $3x10^{-4}M$. Controls were identical but with no added nucleotide. Slices of liver and hepatoma were 0.33 mm thick; 350 mg of slices were used in the incubations,

carried out at 37° C for two hours in a metabolic shaker at 100 oscillations per minute.

All experiments involved male rats of the ACI/f Mai strain, maintained on Purina rat chow. Two strains of transplantable hepatoma were studied: 1) Morris hepatoma #3924A, a poorly-differentiated, rapidly-growing adenocarcinoma of liver, and 2) Morris hepatoma #9121, a so-called "minimal-deviation" hepatoma, which grows slowly and is very active in sterol synthesis. Both hepatoma types were implanted in the right thighs of the host animals.

Digitonin-precipitable sterols, fatty acids, and metabolically-generated $CO_2$ were assayed as described previously (1). Radioactivity was determined with a Packard liquid scintillation spectrometer, model 3390, using a scintillation fluid constructed as outlined elsewhere (5).

## RESULTS AND DISCUSSION

Table 1 compares the effects of cAMP and dibutyryl cAMP on incorporation of acetate-2-$^{14}C$ into cholesterol (as digitonin-precipitable sterol), fatty acid, and $^{14}CO_2$ by Morris hepatoma #3924A, the liver of the tumor-bearing animal, and the liver of a comparable, tumor-free animal. The tumor, in contrast to the normal liver from the tumor-free animal, showed no suppression in sterologenesis or fatty acid synthesis with either cyclic nucleotide. Both normal liver and, to a lesser extent, the liver from the tumor-bearing animal, demonstrated inhibition in lipogenesis resulting from incubation with both cAMP and dibutyryl cAMP. In no instance was $^{14}CO_2$ evolution depressed. Table 2 shows the results of a similar experiment on Morris hepatoma #9121. The results are qualitatively similar. The capacity for regulation of lipogenesis by cAMP is absent in both the undifferentiated (#3924A) and the "minimal deviation" (#9121) hepatomas.

The observed failure of cAMP to suppress lipogenesis in hepatomas contrasts with our previously reported findings in normal liver (1). Taken together, these data correlate well with those of Granner (6), demonstrating the absence of cAMP binding protein in hepatoma cells and its presence in normal liver cells. Presumably absence of this binding protein would prevent the normal response to cAMP.

TABLE 1

| | DPS* | FA | $^{14}CO_2$ |
|---|---|---|---|
| Normal liver-(tumor-free animal) | | | |
| Control | 21717±2421 | 7596±1408 | 23956±1854 |
| Dibutyryl cAMP | 5248±803 | 690±112 | |
| cAMP | 5578±839 | 936±244 | 29938±15981 |
| Morris hepatoma #3924A. | | | |
| Control | 27805±5343 | 1985±354 | 15885±1210 |
| Dibutyryl cAMP | 27762±11555 | 1699±1760 | 17252±5504 |
| cAMP | 23461±4949 | 1739±499 | 17171±7128 |
| Liver-(tumor-bearing animal) | | | |
| Control | 3834±881 | 921±197 | 22184±4337 |
| Dibutyryl cAMP | 2117±450 | 542±136 | 18640±1275 |
| cAMP | 2569±457 | 468±79 | 34997±1050 |

Table 1: Effects of cAMP and dibutyryl cAMP on incorporation of $^{14}C$-acetate into digitonin-precipitable sterol, fatty acid and $^{14}CO_2$ by tissue slices of liver and Morris hepatoma #3924A. Data represents cpm incorporated by 350 mg slices/2 hr., as mean ± S.E. of triplicate analyses. Relative incorporation of acetate-2-$^{14}C$ into these three fractions may be obtained by multiplying cpm value for fatty acids by 4, and for $CO_2$ by 10. Final concentrations of cAMP and dibutyryl cAMP are as indicated in the text.

* Digitonin-precipitable sterol (predominantly cholesterol)

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TABLE 2

| | DPS* | FA | $^{14}CO_2$ |
|---|---|---|---|
| Normal liver-(tumor-free animal) | | | |
| Control | 12190±2462 | 8342±715 | 24072±7780 |
| cAMP | 5745±766 | 1333±182 | 20641±3996 |
| Morris hepatoma #9121 | | | |
| Control | 17240±462 | 1827±472 | 7251±1105 |
| cAMP | 22376±2585 | 1496±122 | 7518±672 |
| Liver-(tumor-bearing animal) | | | |
| Control | 6034±667 | 2216±770 | 41913±5223 |
| cAMP | 3895±906 | 831±268 | 37655±8969 |

Table 2: Effects of cAMP on incorporation of $^{14}C$-acetate into digitonin-precipitable sterol, fatty acid, and $^{14}CO_2$ by tissue slices of liver and Morris hepatoma #9121. Data represents cpm incorporated by 350 mg. of slices/2 hr., as mean ± S.E. of triplicate analyses. Relative incorporations of acetate-2-$^{14}C$ into these three fractions may be obtained as in the legend of Table 1.

* Digitonin-precipitable sterol (predominantly cholesterol)

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