SYNTHESIS OF LIPIDS, CHOLESTEROL PRECURSORS AND CHOLESTEROL IN GLYCOGEN-FREE AND GLYCOGEN-SYNTHESIZING SUBLINES OF THE EHRLICH ASCITES TUMOR.

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Summary

The conversion of mevalonic acid into cholesterol was investigated in two strains of the Ehrlich ascites tumor. The cells of the G+ strain contain glycogen in the nucleus and cytoplasm and synthesize glycogen from added glucose. The cells of the GØ strain do not show this quality. The rate of decarboxylation of mevalonic acid which reflects synthesis of cholesterol and its precursors is low in G+ cells and independent of the time interval between inoculation of the tumor and aspiration. In GØ cells the rate of mevalonic acid decarboxylation rises linearly, starting the fourth day following inoculation of the tumor. Carbon balances for the utilization of [^{14}C] glucose show that the rate of lipid formation is higher in GØ cells.

Recently Katz et al.have published a paper in this journal dealing with a strain of Ehrlich ascites cells, H, containing a fraction of cells which are able to synthesize and to store glycogen. In contrast another strain, HL, does not form any glycogen at all though it contains the necessary complement of enzymes (1). In this context some of our previously unpublished observations may be of interest. We wish to present data concerning the synthesis of cholesterol and its precursors by two well defined Ehrlich ascites strains (2-10).One of these strains, G+, consists of cells which are able to synthesize (10) and to

store (2, 5-8) glycogen, G+, and the other, GØ, shows no glycogen accumulation in any phase of growth.

METHODS

Ascites Cells. Two strains G+ and GØ (6) were propagated by i.p. injection into NMRI mice bread under SPF conditions and fed ad libitum with tap water and Altromin (11) diet.

Radiochemicals and Liquid Scintillation Counting. Radioisotopes were products of the Radiochemical Centre (Amersham,U.K.): 1-[^{14}C]mevalonic acid lactone (6.85 Ci/mole), 2-[^{14}C]mevalonic acid lactone (5.85 Ci/mole), [^{14}C]D-glucose (U) (3 Ci/mole). Absolute radioactivity countings were performed in homogeneous solutions (Protosol, NEN Chemicals, Dreieichenhain, G.F.R.) (12).

Decarboxylation of 1-[^{14}C]Mevalonic Acid. Incubations were performed in Warburg-vessels under gentle shaking at 37° for 2.5 hours. Incubates contained in a total volume of 5 ml of Hanks' balanced salt solution (pH 7.4) 5-25 x 10^7 cells and 1 µCi of 1-[^{14}C]mevalonic acid lactone (preincubated with equimolar amount of KOH for 30 min at 37°). The center well contained 0.5 ml of Protosol and the side arm contained 1 ml of phosphoric acid (85 %). Incubations were stopped by mixing the phosphoric acid with the incubate. After 14 hours at room temperature the Protosol solution which had absorbed the $^{14}CO_2$ was removed and mixed with an additional 2 ml of Protosol and 10 ml of scintillation fluid (12).

Lipid Extracts. Cells were incubated in conical flasks as described above with 2 µCi of 2-[^{14}C]mevalonic acid lactone (preincubated with equimolar amount of KOH for 30 min at 37°) for 1 hour. Incubations were stopped by adding 1 ml of cold (4°) trichloroacetic acid. The precipitate was mixed with 2 ml of 0.5 M NaOH and extracted with chloroform/methanol 2 : 1. The extract was evaporated in vacuo and dissolved in 1 ml of ethanol. Lipid extracts were mixed with unlabeled squalene, lanosterol and cholesterol and aliquots were used for radiochromatography.

Chromatography. Lipid extracts were chromatographed on TLC sheets Silica gel 60 (Merck, Darmstadt, G.F.R.). Solvent system was benzene/acetic acid ester/acetic acid 30 : 5 : 1. Radioscans were made as described in a previous paper (13).

Utilization of [^{14}C]Glucose. Ten days following inoculation of the tumor 5 µCi of [^{14}C]glucose were injected i.p. into mice bearing G+ or GØ tumor. 30 min following injection the cells were harvested, washed free of ascitic fluid and approximately 10^7 cells were denatured with 2 ml of cold (4°) TCA. The precipitate was extracted by chloroform/methanol as described above. The residue was dissolved in Protosol. The TCA extract was mixed with 10 mg of glycogen (Serva, Heidelberg, G.F.R.) and 2 vol. of ethanol were added. The precipitated glycogen was collected by centrifugation and dissolved in Protosol. This fractionation was carried out 6 times for each tumor.

Results

Decarboxylation of Mevalonic Acid in Ehrlich Ascites Cells.

Tables I and II show that a linear relationship exists between $^{14}CO_2$ production from 1-[^{14}C]mevalonic acid and the cell number indicating that dilution of the radioactively labeled precursor by endogeneous mevalonic acid can be neglected in both cell types. Therefore, the rate of $^{14}CO_2$ production from 1-[^{14}C]mevalonic acid can be regarded as the rate of conversion of mevalonic acid into cholesterol and its precursors.

Differences in the Rate of Decarboxylation of Mevalonic Acid between G+ and GØ Cells. Tables I and II show that by day 10 of tumor growth the GØ strain has a higher rate of mevalonic acid decarboxylation than does the G+ strain. Figure 1 presents the changes in the rate of decarboxylation during the course of tumor growth for the two strains. Between day 4 and day 12, the rate of decarboxylation by G+ cells remains nearly constant. During this same period, the decarboxylation rate of the GØ cells is increasing linearly. A single linear extrapolation of the two graphs indicates that the decarboxylation rates would be equal on the third day.

TABLE I

$^{14}CO_2$ production by GØ cells during incubation for 2.5 hours with 1-[^{14}C]mevalonic acid. Cells were harvested 10 days following inoculation.

Cell number per incubate (X 10^{-7})	$^{14}CO_2$ production [dpm x 10^{-3}]	$^{14}CO_2$ production [dpm x 10^{-3}]/10^5 cells
13.6	219.4	161
9.0	140.4	156
6.8	101.2	149

TABLE II

$^{14}CO_2$ production by G+ cells during incubation for 2.5 hours with 1-[^{14}C]mevalonic acid. Cells were harvested 10 days following inoculation.

Cell number per incubate (X 10^{-7})	$^{14}CO_2$ production [dpm x 10^{-3}]	$^{14}CO_2$ production [dpm x 10^{-3}]/10^5 cells
24.6	165.5	67
16.4	98.9	60
12.3	84.1	68

Relative Amounts of Cholesterol Precursors in Ehrlich Ascites Cells. A typical radioscan of lipid extracts from ascites cells incubated with 2-[^{14}C]mevalonic acid is shown in Figure 2. Squalene and lanosterol accumulated and could be easily identified by cochromatography of the unlabeled substances. Cells of the GØ and G+ strain showed no differences in the relative distribution of these precursors.

Utilization of ^{14}C-Glucose. Table III shows the uptake of ^{14}C into the glycogen- and lipid-fraction of the two cell strains following an in vivo pulse of 30 min with [^{14}C]glucose. The results demonstrated in Table III show that added glucose is utilized by G+ cells in about the same ratio for glycogen and lipid synthesis. In contrast GØ cells utilize glucose to a much higher degree for lipid synthesis. It is assumed that the 5 % incorporation of radioactively labeled glucose into the glycogen fraction of GØ cells reflects contamination rather than incorporation.

DISCUSSION

The absence of glycogen is a characteristic of certain

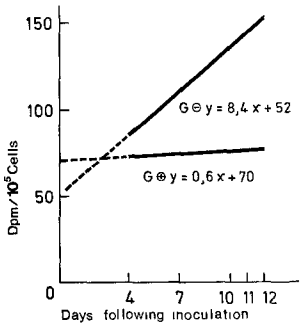

FIGURE 1

Ehrlich ascites cells (GØ and G+) were harvested at the days indicated and the rate of 1-[^{14}C]mevalonic acid decarboxylation was estimated as described under methods. The values of five samples taken at each day indicated were used for regression analysis. Linear regression coefficient for GØ cells was 0.7416.

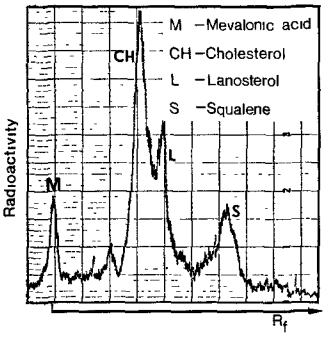

FIGURE 2

Radioscan of lipid extracts from Ehrlich ascites tumor cells which were incubated with 2-[^{14}C]mevalonic acid.

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strains of the Ehrlich ascites tumor (14). According to Baba and Tsuiki (15), this feature is caused by the presence of a factor of presumably protein nature in these tumor cells. It inhibits the activation of glycogen synthase (UDPglucose: glycogen 4-α-glucosyltransferase, EC 2.4.1.11). Glycogen synthase has been found to be present in glycogen-free Ehrlich ascites

TABLE III

Uptake of [^{14}C]glucose into various fractions of G+ and GØ cells at the seventh day following inoculation.

Fraction	G+ Tumor [dpm/10^6 cells]	% of Total	GØ Tumor [dpm/10^6 cells]	% of Total
Glycogen	102.6 ± 2.1	21.2	17.9 ± 3.0	5.1
Lipid	164.8 ± 2.2	34.1	181.0 ± 10.3	51.8
Rest	216.5 ± 2.3	44.7	150.7 ± 7.4	43.1
Lipid/Glycogen	1.61		10.55	

cells in an amount sufficient to conduct glycogenesis at a substantial rate (15). Ehrlich ascites strains containing and synthesizing glycogen (1,8,10) are apparently lacking the inhibitory factor described by Baba and Tsuiki (15). The comparison of glycogen-free and glycogen-producing Ehrlich ascites cells *in vitro* with respect to the formation of glucose metabolites of low molecular weight did not reveal much difference (1). The data presented here, however, show a significant difference between G+ and GØ cells in the TCA-insoluble material produced from exogeneous [^{14}C]glucose. While G+ cells converted a major part of the labeled glucose into glycogen, the glycogen-free cells utilized glucose preferentially for lipid synthesis. This favours the idea that there exists a competition between glycogen synthesis and lipid synthesis in Ehrlich ascites cells. These *in vivo* findings are paralleled by differences in the rate of formation of cholesterol precursors as determined *in vitro*. G+ cells exhibit a relatively low but constant rate which is independent of the transplantation age of the tumor from day four to day twelve. In sharp contrast, the rate of formation of

cholesterol precursors by GØ cells increases linearly from day four to day twelve after tumor transplantation. Interestingly, the glycogen accumulation in G+ cells starts after day three, when the two strains may start to differ in their rate of decarboxylation (8). Furthermore, the growth rate of Ehrlich ascites cell populations begins to decline between day three and four after transplantation (16), and G2 and M-arrested cells start to accumulate (17). The coincidence of these changes in the proliferation pattern and the metabolic activities of the tumor cells which apparently cannot be influenced by hormonal control mechanisms (1) deserves further investigation.

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