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Regulation of cholesterol biosynthesis by normal and leukemic (L_2C) guinea pig lymphocytes

[cholesterol exchange/hydroxymethylglutaryl-CoA reductase (NADPH)/Arrhenius plots/membrane state/neoplastic transformation]

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ABSTRACT **The cholesterol production of guinea pig leukemic (L_2C) lymphocytes proceeds at greater than 30 times the rate found in normal cells. Fatty acid biosynthesis is also enhanced in L_2C cells. Exposure of L_2C cells to cholesterol/lecithin liposomes does not depress their sterol biosynthesis, in contrast to the behavior of normal lymphocytes [Philippot, J. R., Cooper, A. G. & Wallach, D. F. H. (1975) *Biochim. Biophys. Acta* 406, 161–166]. However, 25-hydroxycholesterol, an inhibitor of hydroxymethylglutaryl-CoA reductase (NADPH) [mevalonate: $NADP^+$ oxidoreductase (CoA-acylating), EC 1.1.1.34], the rate limiting enzyme in cholesterogenesis, and 25-hydroxycholecalciferol, a biologically potent form of vitamin D_3, block sterol biosynthesis of both normal and L_2C lymphocytes [Philippot, J. R., Cooper, A. G. & Wallach, D. F. H. (1976) *Biochem. Biophys. Res. Commun.* 72, 1035–1041]. Moreover, both cell types exchange cholesterol equivalently with cholesterol/lecithin liposomes. The only difference in sterol biosynthesis observed between the two cell types is in the temperature response of the enzyme. Arrhenius plots of this enzyme activity exhibit a prominent discontinuity at about 24° in the case of normal cells, but none in the case of L_2C. The activation energies for L_2C cells and normal cells, above the normal cell transition temperature, were not significantly different. All of the data suggest that the regulatory defect in L_2C lymphocytes arises from a deficiency in these cells' internal membranes.**

Normal cells typically exhibit a suppression of sterol biosynthesis after exposure to external cholesterol (1). In contrast, neoplastic cells almost universally lack this response and may synthesize cholesterol at rates so rapid as to lead to cellular cholesterol enrichment (1–3). These metabolic deficiencies occur also in leukemic cells (1–4). Indeed, as shown in an earlier study (5) leukemic L_2C guinea pig lymphocytes synthesize cholesterol *in vitro* at a rate 30–40 times that of normal cells, and unlike normal lymphocytes, do not decrease cholesterol production upon exposure to cholesterol phospholipid liposomes. However, both cell types showed equivalent suppression of *in vitro* sterol biosynthesis after exposure to androstane nitroxide, an electron spin resonance probe, and 25-hydroxycholesterol, a potent inhibitor of HMG CoA-reductase [hydroxymethylglutaryl-CoA reductase (NADPH), mevalonate: $NADP^+$ oxidoreductase (CoA-acylating), EC 1.1.1.34], the endoplasmic reticulum enzyme that acts as the principal regulator of cholesterogenesis (6).

We have now extended our earlier studies (5), in an effort to better define the mechanism(s) responsible for defective regulation of cholesterol biosynthesis in L_2C cells.

MATERIALS AND METHODS

Materials. Androstane nitroxide (17β-hydroxy-4′,4′-dimethylspiro [5α-androstan-3,2′-oxazolidin]-3′-yloxyl) was purchased from Syva Corp. (Palo Alto, Calif.), 5-cholesten-3β-25-diol (25-hydroxycholesterol) from Steraloids Inc. (Wilton, N.H.), egg lecithin from Lipid Products (South Nutfield, England), cholesterol (analytical grade) from Sigma Chemical Co. (St. Louis, Mo.), Hanks' balanced salt solution, Hepes buffer [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid], phosphate-buffered saline, and RPMI 1640 from Gibco (Grand Island, N.Y.). Aquasol 2, [1-^{14}C]acetate, [^{3}H]leucine, [^{14}C]cholesterol, [^{3}H]cholesterol, DL-[3-^{14}C]hydroxymethylglutaryl CoA, and [^{3}H]mevalonate were purchased from New England Nuclear (Cambridge, Mass.) All other reagents were of analytical grade.

Cells. The L_2C leukemia, which arose spontaneously in a strain 2 guinea pig (7) was serially passaged in syngeneic or semisyngeneic guinea pigs (8) for this study. L_2C cells have been recently characterized as B cell lymphoblasts (9). Normal and L_2C leukemia were harvested as previously described (5). The cells, washed in Hanks' balanced salt solution, were washed once in RPMI 1640, 15 mM in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.6, and containing 100 units of penicillin and 0.1 mg of streptomycin per ml, then resuspended in this medium and used either immediately or after overnight storage at 4° in the incubation medium.

Analysis of Cholesterol, Fatty Acid, and Protein. Nonradioactive cholesterol was determined by the technique of Parekh and Jung (10) on the digitonin precipitates, by using [^{3}H]cholesterol as an internal standard. Phospholipids were measured with the Bartlett's method (11) after digestion of samples 3 hr at 165° with 0.5 ml of 10 M perchloric acid. Proteins were measured according to Lowry *et al.* (12).

Inhibition Studies. To study the effects of various inhibitor concentrations on cholesterol, fatty acid, or protein synthesis, we incubated 4 to 6 × 10^6 cells for 2 hr at 37° with different concentrations of inhibitor, before addition of [1-^{14}C]acetate or [^{3}H]leucine (10 μl) to a total volume of 2 ml. For experiment on reversibility of inhibition, the cells were washed three times after this step, by using 5 ml phosphate-buffered salt solution pH 7.4 (4°) each time, and finally resuspended in RPMI 1640. Incubation was then continued for up to 4 hr at 37°.

Stock solutions of 25-hydroxycholesterol and androstane nitroxide (10 mM) in ethanol were diluted so as to maintain the final concentration of ethanol below 0.5% (vol/vol). To measure incorporation of [1-^{14}C]acetate into cellular lipids, we used 18 μCi per sample (specific activity was 32 Ci/mol). To measure protein synthesis, we diluted our stock of [^{3}H]leucine (60 Ci/mol) 5-fold with RPMI 1640 before addition to the sample.

After incubation, cell viability was assayed microscopically by trypan blue exclusion (viability—80 to 90% trypan blue-negative cells). To determine incorporation of labeled acetate, we harvested cells by using glass filters (93 LAH; Reeve Angel, Clifton, N.J.) and washed them four times with 10 ml of ice-cold, phosphate-buffered saline, pH 7.4. Then about 10^5 dpm

Abbreviation: HMG CoA, hydroxymethylglutaryl-CoA.
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of [^{3}H]cholesterol was added as internal standard. Cholesterol and fatty acids were measured as described (13). After saponification and extraction with petroleum ether, cholesterol was collected as the digitonin-precipitable fraction. The aqueous phase was brought to pH less than 3 with concentrated HCl and extracted again with petroleum ether. The second ether extract, containing the fatty acids, was dried before assaying for radioactivity. For the assay of [^{3}H]leucine incorporation, we precipitated the protein in an aliquot of cell suspension with ice-cold trichloroacetic acid (5% final concentration), washed at 3000 rpm (20 min, 4° Sorvall GLC-2, rotor HL4) with iced 5% trichloroacetic acid before the radioactivity analyses. Radioactivity was measured with a Packard Tri-Carb (3320) scintillation spectrometer and 10 ml of Aquasol 2 (New England Nuclear Corp.) as scintillation mixture.

Liposomes. Lecithin/cholesterol liposomes (molar ratio of 1) were prepared according to the procedure of Batzri and Korn (14). Briefly, 200 μl of lecithin (100 mg/ml) and 200 μl of cholesterol (50 mg/ml) including 13.5 μCi of [^{14}C]cholesterol, all in organic solvents, were first dried under a stream of nitrogen and then for 1 hr under a vacuum. The lipids were dissolved in ethanol, taken to dryness, and dissolved in minimum volume of ethanol. This solution was rapidly injected through a Hamilton syringe into 80 ml of RPMI 1640 maintained at room temperature. Before use, the suspension was centrifuged 20 min at 48,000 × *g* to remove large aggregates.

In exchange experiments, cells were incubated directly in the liposome suspension at 37°, harvested at different times after three washes with phosphate-buffered salt solution at 4° (1000 rpm, 8 min, 4°, in Sorvall GLC-2 centrifuge with rotor HL4). Radioactivity was then assayed after addition of 10 ml of Aquasol 2 to the sample. Protein, phospholipid, and nonradioactive cholesterol were determined before and after contact with liposomes.

Temperature-Variation of HMG CoA-Reductase Activity. Cells were washed two times with NaCl (0.9%) and suspended in buffer [50 mM potassium phosphate (pH 7.4), 5 mM dithiothreitol, 1 mM EDTA] to get 1–2 mg of protein per ml. Cells were frozen in liquid nitrogen and stored at −80° before use.

To study the influence of temperature on HMG CoA-reductase activity, we thawed cell suspensions at room temperature, sonicated them for 5 s with the microtip of a Sonic 300 Dismembranator (Artek Systems Co, Farmingdale, N.Y.) at power step 30, and activated the enzyme for 10 min at 37°. HMG CoA-reductase activity was measured according to Kandutsch and Chen (15). Aliquots of 50 μl were incubated for 30–60 min at different temperatures in a final volume of 0.2 ml containing 100 mM potassium phosphate (pH 7.4), 5 mM dithiothreitol, 0.25 mM EDTA, 2 mM glucose 6-phosphate, 2.5 mM NADP, 0.6 unit glucose-6-phosphate dehydrogenase, and 16 nmol of [3-^{14}C]HMG CoA (26.2 Ci/mol). The [^{14}C]mevalonate formed was isolated by thin-layer chromatography and assayed, by using an internal standard of [^{3}H]mevalonate to correct for incomplete recovery. Not more than 3% of HMG CoA-reductase activity was solubilized (nonsedimentable at 10^7 × *g*·min). Three different preparations of normal and L_2C cells were tested.

RESULTS

Characteristics of Cholesterol and Fatty Acids Biosynthesis by Normal and Neoplastic Guinea Pig Lymphocytes. Table 1 shows that L_2C lymphocytes synthesize cholesterol at 30–40 times the rate characteristic of normal cells. This correlates with an important enhancement of HMG CoA-reductase activity and an accumulation of cholesterol in the cells (32 nmol/mg of protein in L_2C cells versus 15 nmol/mg of protein in normal lymphocytes).

Table 1. Characteristics of normal and neoplastic (L_2C) guinea pig lymphocytes

Sample	Normal	L_2C
Protein (mg/10^9 cells)	82.2(3)* [72–90]†	93.3(6) [81–122]
Phospholipids (μmol/10^9 cells)	4.75(3) [3.95–5.55]	10.4(3) [9.9–12.4]
Cholesterol (μmol/10^9 cells)	1.2(3) [0.9–1.3]	3.0(6) [2.6–4.0]
Fatty acid biosynthesis (nmol [^{14}C]acetate incorporated per 10^9 cells/hr)	7.8(4) [4.8–11.0]	208(9) [63–576]
Cholesterol biosynthesis (nmol [^{14}C]acetate incorporated per 10^9 cells/hr)	2.9(4) [1.2–4.4]	104(10) [60–146]
HMG CoA-reductase activity (pmol [^{14}C]HMG CoA incorporated/min per mg protein)	11.8(3) [9–13.5]	87.5(4) [77–105]

* Parentheses show the number of determinations in duplicate.
† Figures in brackets give the range.

Table 1 also documents that L_2C cells synthesize fatty acids at 20–30 times the normal rate. This finding may bear on the anomalously high phospholipid content of the neoplastic lymphocytes (111 nmol of lipid phosphorus per mg of protein in L_2C cells versus the normal value of 57 nmol/mg of protein).

Inhibitory Effects of 25-Hydroxycholesterol and Androstane Nitroxide. We found earlier (5) that androstane nitroxide was an even more potent inhibitor of sterol biosynthesis by L_2C cells than 25-hydroxycholesterol, when these agents were added as sonicated suspensions. Under these conditions, half-maximal inhibition occurred with 30–50 μM androstane nitroxide and 0.3–0.5 mM 25-hydroxycholesterol. However, quantitatively different results are obtained when the inhibitors are presented in ethanolic solution.

Figs. 1 and 2 demonstrate the effects of the inhibitors, added in ethanolic solution [final ethanol concentration 0.5% (vol/vol)]

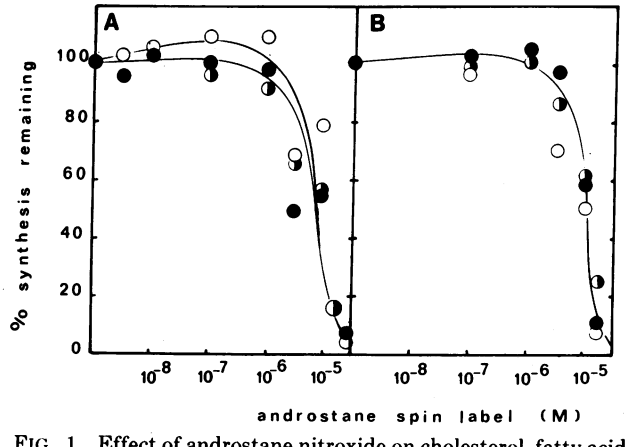

FIG. 1. Effect of androstane nitroxide on cholesterol, fatty acid, and protein biosynthesis by normal (*A*) and leukemic (*B*) guinea pig lymphocytes. Cells were incubated for 2 hr at 37° with different concentrations of inhibitor. Then 18 μCi of [^{14}C]acetate or 2.5 μCi of [^{3}H]leucine was added and incubation was continued for up to 4 hr at 37°. The incorporation of both precursors into cholesterol (●), fatty acid (○), or protein (◑) was measured as indicated in *Materials and Methods*. Each point represents the mean of experiments carried out in duplicate on three different cell populations (1 to 2 × 10^6 cells per ml).

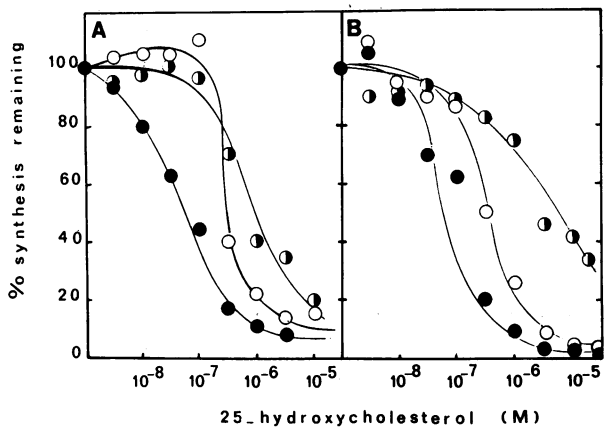

FIG. 2. Effect of 25-hydroxycholesterol on cholesterol (●), fatty acid (○), and protein biosynthesis (◑) by normal (*A*) and leukemic (*B*) guinea pig lymphocytes. Protocol and chemical determinations as in Fig. 1.

on cholesterol, fatty acid, and protein biosynthesis. It is evident that androstane nitroxide inhibits all three biosynthetic processes to the same extent and that it has an equivalent action on normal lymphocytes (Fig. 1*A*) and L_2C cells (Fig. 1*B*). The concentrations for half-maximal inhibition (8–80 μM) are similar to those observed before (5).

In contrast, when 25-hydroxycholesterol, in ethanolic solution, is added to either normal lymphocytes (Fig. 2*A*) or L_2C cells (Fig. 2*B*) a strong suppression of cholesterol biosynthesis occurs at low concentrations of inhibitor. The concentration for half-maximal inhibition, 80 nM, (1 to 2 × 10^6 cells per ml) corresponds to that observed for L cell fibroblasts (6). At higher concentrations, fatty acid biosynthesis is also inhibited with half-maximal suppression at 0.5 μM (1 to 2 × 10^6 cells per ml), but protein synthesis is little affected. Thus, at 0.5 μM 25-hydroxycholesterol, the synthesis of cholesterol is reduced by 80–85% in the two cell types, fatty acid synthesis by 50–60%, and protein synthesis by 15–20%. These values hold for cell concentrations of 1 to 2 × 10^6/ml. When the numbers of cells were increased to 3.2 × 10^6/ml and 5 × 10^6/ml, the inhibitor concentrations for half-maximal suppression of cholesterol biosynthesis rise to 0.3 and 0.45 μM, respectively. A corresponding increase was observed in the concentration for half-maximal fatty acid biosynthesis.

Reversibility of Inhibition. As shown in Table 2, the inhibition produced by exposure of normal and L_2C cells to 25 μM androstane nitroxide or 25-hydroxycholesterol for 2 hr at 37°, can be largely reversed by washing the cells at low temperature. The synthetic rates for cholesterol, fatty acids, and proteins of cells thus treated were 86, 72, and 96% of the control rates for androstane nitroxide and 80, 100, and 95%, respectively, for 25-hydroxycholesterol (Table 2; columns B). However, the wash step somehow modifies the susceptibility of the cells to the action of 25-hydroxycholesterol during the second incubation with inhibitor (Table 2; columns A), because the degrees of inhibition, 41, 37, and 21% for cholesterol, fatty acid, and protein synthesis, respectively, are much lower than those observed at this inhibitor level under standard conditions (Fig. 2), namely 99, 98, and 68%, respectively. This effect is not observed with androstane nitroxide.

Exchange of Cholesterol between Lecithin/Cholesterol Liposomes (Molar Ratio of 1) and Whole Cells. Cholesterol, presented in the form of lecithin/cholesterol liposomes, can inhibit cholesterol biosynthesis *in vitro* by normal lymphocytes but not by L_2C cells (5). Since Heiniger *et al.* (16) have shown that plasma membrane cholesterol of L cell fibroblasts must be maintained within narrow limits to allow endocytosis, and because L_2C cells are cholesterol-enriched, we searched for a possible defect in cholesterol exchange between L_2C cells and liposomes. Such a defect could explain the deficient response of L_2C cells to cholesterol/lecithin liposomes.

Table 3 presents the principal results of the exchange experiments. These exchange values correspond to net uptake of radioactive cholesterol into the cells from prelabeled liposomes. The cholesterol/phospholipid molar ratio of the liposomes was maintained at 1.0, approximately the same as the plasma membrane ratio (2), to avoid gross cholesterol gradients.

After a 3 hr incubation, i.e., within the period where inhibition of cholesterol synthesis occurs in normal cells (5), the cholesterol content of normal cells had increased by 20% and that of L_2C cells by 28%. However, the amount of cholesterol exchanged was identical for normal and L_2C cells, as was the relative initial velocity. The unresponsiveness of the L_2C cells can thus not be attributed to a deficient sterol-exchange process.

Temperature-Activity Relation of HMG CoA-Reductase of Normal and L_2C Cells. The results of these studies are given in Fig. 3. Measurements were taken after 10 min preincubation at 37°. The data are given in the form of Arrhenius plots, which were established by linear regression analyses. In the case of three L_2C cell-preparations, only a single slope was obtained with a correlation coefficient, in each case, of greater than −0.99. However, in the case of normal lymphocytes the semilogarithmic plot of enzyme activity versus 1/T invariably gave two slopes, all with correlation coefficients greater than −0.98. The transition temperatures suggested by the intercept of the

Table 2. Recovery of cholesterol, fatty acid, and protein biosynthesis in guinea pig lymphocytes after 2 hr exposure to androstane nitroxide and 25-hydroxycholesterol*

	Synthetic rate					
	Cholesterol		Fatty acid		Protein	
Inhibitor (25 μM)	A	B	A	B	A	B
Androstane nitroxide	24 [14–40]	86 [79–110]	26 [10–40]	72 [56–83]	52†	96†
25-hydroxycholesterol	59 [48–60]	80 [78–81]	63 [56–70]	100†	79 [78–80]	95 [73–117]

* The synthetic rates are expressed as the percent of control cell values after incubating and washing in the absence of sterol inhibitors. These syntheses correspond to material labeled with [^{14}C]acetate or [^{3}H]leucine during a 2-hr-incubation period in the presence of inhibitors 25 μM (A) or in their absence (B). In both cases, cells were preincubated 2 hr with 25 μM inhibitor at 37°, then washed three times at 4° with ice-cold incubation medium. Values represent averages [ranges] of the results of five different experiments for androstane nitroxide, and the results for three experiments for 25-hydroxycholesterol, each sample in duplicate, except for values indicated by † where only one cell preparation was tested. The mean cell concentrations were 1.4 × 10^6/ml of normal cells and 1.9 × 10^6/ml of L_2C cells.

Table 3. Characteristics of cholesterol exchange between lecithin/cholesterol liposomes and whole cells*

	Normal lymphocytes	L_2C
Specific activity in the cells after 3 hr (Ci/mol)	0.12 [0.08–0.17]†	0.10 [0.06–0.135]
Relative initial velocity (dpm/min per μmol cellular cholesterol)	2.55×10^5 [$2.2\ 10^5$–$2.9\ 10^5$]	2.65×10^5 [$2.6\ 10^5$–$2.8\ 10^5$]
Exchangeable cholesterol (percent of total cholesterol)	21.6 [13.9–29.2]	22.2 [11.2–29]

* Cells (3×10^6/ml) were incubated in a lecithin/cholesterol liposome suspension (300 μM), prepared according to the technique of Batzri and Korn (14), containing 3×10^5 dpm [^{14}C]cholesterol/ml with a final specific activity in the medium of 0.56 Ci/mol. At different times, duplicate samples were harvested, cells collected by centrifugation, and they were washed 3 times with 5 ml of ice-cold phosphate-buffered salt solution, pH 7.4. Total cholesterol and radioactive cholesterol were measured as indicated in *Materials and Methods* on cells and aliquots of incubation medium. Values are the mean of four different experiments for L_2C and three experiments for normal cells. Cholesterol exchange was calculated as in Bruckdorfer *et al.* (17).

† Figures in brackets give the range.

two slopes were 24°, 24°, and 20° in the three samples. We computed an average activation energy of 30 kcal·mol^{-1} (range 27.5–31.4) for L_2C cells and, for normal cells, 23 kcal·mol^{-1} (range 20.7–26.1) above 20° and 49 kcal·mol^{-1} (range 46.6–51.9) below the transition temperature. The difference between the values obtained for normal cells above the transition and the L_2C value is not significant ($P \geq 0.05$).

DISCUSSION

Defective regulation of cholesterol biosynthesis and overproduction of this sterol are among the most pervasive biochemical abnormalities of tumor cells (1–3) and there is evidence that cholesterol overproduction increases its proportions in intracellular membranes (2). As noted before (18), these circumstances could lead to profound and pleiomorphic functional deviations, because cholesterol acts to modify the state of membrane lipid domains and the properties of the enzymes located therein. This has already been documented for several membrane enzymes (19, 20), which can be inactivated by excessive concentrations of cholesterol. However, no such information exists for HMG CoA-reductase, a membrane enzyme that is the key regulatory enzyme of cholesterogenesis (21).

Cholesterogenesis in L_2C cells, (1–3), is abnormal in terms of both intrinsic control and regulation by external cholesterol. We therefore asked the following questions: (*i*) is the responsiveness of HMG COA-reductase different in normal and L_2C lymphocytes? (*ii*) Are L_2C cells deficient in their ability to take up cholesterol? (*iii*) Is the membrane state of the HMG CoA-reductase different in normal and L_2C cells?

That the anomalous cholesterol metabolism of hepatomas might be due to an abnormal HMG CoA-reductase has been satisfactorily excluded (22–24). Our studies, utilizing 25-hydroxycholesterol as an inhibitor specific for HMG CoA-reductase, lead to the same conclusion for L_2C: (*i*) L_2C cells are

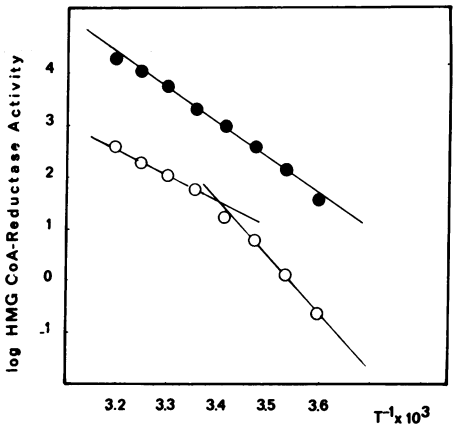

FIG. 3. Typical Arrhenius plots of hydroxymethylglutaryl CoA-reductase activity for normal (○) and L_2C cells (●). The straight lines are fitted by linear regression. The regression coefficient for L_2C was −0.991. The coefficients for normal cells were −0.987 and −0.98, respectively, for the slopes above and below 20°.

inhibited in an identical fashion to normal cells by 25-hydroxycholesterol; (*ii*) a decrease of the amount of inhibitor proffered per cell equivalent diminishes inhibition identically in the two cell types; (*iii*) inhibition can be identically reversed by washing the cells. The anomalous cholesterol metabolism of L_2C cells can therefore not derive from an unresponsive regulatory enzyme, a lesser number of inhibitor binding sites, or a more rapid recovery from an inhibitory stimulus.

Also, because normal and L_2C cells exhibit identical exchange kinetics after exposure to lecithin/cholesterol liposomes, the abnormal cholesterol metabolism of L_2C cells cannot be ascribed to an anomalous penetration of extrinsic sterol.

The membrane state of HMG CoA-reductase, is not easily approached, but our data on the thermal responsiveness of this enzyme in normal and L_2C cells give some clues. The Arrhenius plots obtained with normal cells show a clear discontinuity at about 24°. The transition temperature for normal lymphocytes as well as their activation energies above and below the transition temperature, i.e., 23 kcal·mol^{-1} and 49 kcal·mol^{-1}, respectively, are very close to the values reported for microsomes from the livers of cholesterol-deficient rats, 19.5 kcal·mol^{-1} and 45.2 kcal·mol^{-1}, respectively (25). The Arrhenius plots for L_2C cells, in contrast, show *no* discontinuity and give an activation energy identical to that of normal lymphocytes above the transition temperature.

Arrhenius-plot discontinuities of an enzyme activity signify cooperative changes of phase within the enzyme or its microenvironment (26). Discontinuities in the temperature range observed for HMG CoA-reductase are characteristic of membrane-associated enzymes, and reflect phase transitions of enzyme-associated acyl chains (26). Moreover, the sharpness of a given discontinuity is a function of the cooperativity of the lipid phase transition. Our data therefore signify that membrane lipids associated with the HMG CoA-reductase of normal cells go through a cooperative phase transition at about 24°, whereas those of L_2C cells do not. The biological significance of a thermotropic phase transition is not necessarily in the circumstance that it can be thermally-induced but also in the fact that transitions induced by thermal shifts at unphysiological temperatures might also be induced at biologically-meaningful temperatures by shifts of, for example, pH or [Ca^{2+}] (18). The Arrhenius data thus point to an unresponsiveness of L_2C cell membranes that may be relevant to the defective regulation of these cells' sterol biosynthesis.

Three hypotheses can account for the lack of HMG CoA-

reductase thermotropism in disrupted L_2C cells: first, the high cholesterol production by these cells may "pollute" HMG CoA-reductase-containing membranes, producing an "intermediate" state of lipid fluidity at all tested temperatures (18). That this can occur has been shown by Sabine and James (25) who demonstrate a disappearance in the Arrhenius discontinuity of hepatic HMG CoA-reductase activity after cholesterol feeding. However, cholesterol enrichment of membrane lipid is expected to alter activation energies above the transition temperature (25, 27) and we do not detect this. Second, the phospholipids associated with the HMG CoA-reductase of L_2C cells might be more unsaturated than those of normal cells, shifting the transition to temperatures too low for enzyme activity measurements. However, acyl chain unsaturation should also change activation energies (see ref. 28). Third, certain proteins can shift and/or modify phase transitions in model phospholipid membranes (18). A search for such candidate proteins in the internal membranes L_2C cells is feasible.

Our data suggest that the defective regulation of cholesterol biosynthesis in L_2C lymphocytes may derive from an abnormal constitution of these cells' internal membranes. It remains to be established how the abnormal production of cholesterol by L_2C cells relates to their high fatty acid biosynthesis, and whether this pathway is also inadequately regulated at the membrane level, as in the case of hepatomas (29).

After completing this study, we discovered (30) that 25-hydroxycholecalciferol, a biologically potent derivative of vitamin D_3, inhibits cholesterol biosynthesis of both normal and L_2C lymphocytes to the same extent as 25-hydroxycholesterol in terms of concentration, but much more rapidly than 25-hydroxycholesterol.

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