Defective 3-ketosteroid reductase activity in a human monocyte-like cell line

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Abstract The human monocyte-like cell line U937, which is a cholesterol auxotroph, does not grow on mevalonate, squalene, or 4,4-dimethyl cholest-7-en-3β-ol. It grows on cholest-7-en-3β-ol and converts it to cholesterol. When deprived of an exogenous source of cholesterol, the cells accumulate 4α-methyl-cholest-8-en-3-one. The cell-free extracts of U937 are also devoid of 3-ketoreductase activity. The present studies indicate that the lesion in cholesterol synthesis by these cells is located at 3-ketosteroid reductase, making this the first report of a deficiency of this enzyme. In contrast, another U937 strain (U937-N) synthesizes cholesterol, does not accumulate 4α-methyl-cholest-8-en-3-one, and has 3-ketosteroid reductase activity. The two strains should be valuable in studies of the regulation of cholesterol metabolism and of the role of cholesterol in membrance structure and function.—**Billheimer, J. T., D. Chamoun, and M. Esfahani.** Defective 3-ketosteroid reductase activity in a human monocyte-like cell line. *J. Lipid Res.* 1987. **28:** 704—709.

Supplementary key words 4α-methyl-cholest-8-en-3-one • macrophage-like U937 cells

Macrophages play an important role in several immunologic processes including phagocytosis, presentation of antigens to T and B cells, and their capacity to destroy tumor cells (1). In addition, lipid-laden macrophages (foam cells) may facilitate the deposition of cholesterol during atherogenesis (2). The human monocyte/macrophage-like cell line U937 is a histiocytic lymphoma which was established by Sundström and Nilsson (3) and adapted for rapid growth in culture by Lackman, Moore, and Metzar (4). Its phenotype represents monocytic cells arrested at a differentiation step close to the myelomonocytic stem cells (3, 4). When stimulated, U937 cells differentiate into macrophage-like cells which have been used as a model of macrophage function (5, 6). We have recently demonstrated a requirement for cholesterol for growth of U937 (7). Although a number of other cholesterol auxotrophs have been isolated from mammalian cells, none have been from an established human cell line and, in most cases, the metabolic defect has not been established. The purpose of these experiments is to determine at what point in the cholesterol biosynthetic pathway the U937 line is deficient. Since U937 requires only cholesterol for growth, then the block must be after the formation of squalene and the determination of the enzymic defect may allow the future use of molecular biological techniques to purify the enzyme. Membrane-bound enzymes of cholesterol biosynthesis have proven extremely refractory to purification by normal methods. Furthermore, Kandutsch, Chen, and Heiniger (8) have demonstrated that cholesterol synthesis in cell culture is regulated by oxysterols such as 25-hydroxycholesterol. A number of oxysterols are intermediates in the biosynthetic pathway of cholesterol (e.g., lanosta-8-en-3, 32 diol), and it has been suggested that one of these or an oxygenated intermediate (e.g., lanosterol epoxide) may be the endogenous regulatory compound (9. 10). A mutant blocked at the enzymic reaction of interest or the subsequent step would be of value in evaluating the endogenous oxysterol hypothesis and in studies of the regulation of steps committed to cholesterol biosynthesis.

EXPERIMENTAL PROCEDURES

Materials

Cholesterol, cholest-7-en-3β-ol, and lanosterol were purchased from Steraloids. 4,4-Dimethyl-cholest-7-en-3β-ol and the corresponding 4α-methyl-sterol were prepared by the methods of Gautschi and Bloch (11) and Neiderhiser and Wells (12), respectively. [30-$^3H_3$]4α-methyl-cholest-7-en-3β-ol and [^{14}C]acetate were obtained from

Abbreviations: PBS, phosphate-buffered saline; FCS, fetal calf serum; GLC-MS, gas-liquid chromatography-mass spectrometry; TLC, thin-layer chromatography.

New England Nuclear. 4α-Methyl-cholest-7-en-3-one and 4α-[30-$^3H_3$]-methyl-cholest-7-en-3-one (5,000 dpm/nmol) were prepared from the corresponding alcohol by oxidation with *tert*-butyl chromate (13). Cholest-8-en-3β-ol was prepared by reduction of the corresponding 8,14 diene as previously described (14). Where necessary, these sterols and lanosterol were purified by high performance liquid chromatography and were >98% pure. Acetate, squalene, mevalonate, isocitrate, isocitrate dehydrogenase, glucose oxidase, NADP, and crystalline bovine serum albumin (BSA) were obtained from Sigma Chemical Company. Triton WR-1339 was from Ruger Chemical Company. Heat-inactivated fetal calf serum (FCS) and RPMI 1640 with glutamine (0.3 g/liter) were obtained from Flow Laboratories.

Cell strain and growth conditions

The human macrophage-like cell line U937 was established by Sundström and Nilsson (3) and has been propagated in a number of laboratories. In the present experiments, those cells designated U937 were a gift from Dr. Hillel S. Koren of Duke University Medical Center and represent a mixed population of U937 cells. A different isolate designated U937-N was cloned by Dr. Robert Newton of E. I. du Pont. Both U937 and U937-N showed the following positive monocyte markers: PM81, LeuM1, and after induction by phorbol myristate, LeuM5 and OKM5. The cells were grown at 37°C in suspension cultures in RPMI 1640 with glutamine (0.3 g/liter) supplemented with 10% heat-inactivated fetal calf serum, 1 mM Na pyruvate, 100 units per ml of penicillin, and 78 units per ml of streptomycin in a humidified 5% CO_2 environment. Growth was monitored by diluting 0.5 ml of the culture with 19.5 ml of phosphate-buffered saline (PBS) and counting the cells in a Coulter Counter.

Lipids were removed from FCS according to the procedure of Rothblat et al., (15) which reduced the cholesterol to <30 ng/ml (7).

Lipid extraction and sterol analysis

About 5×10^8 cells were harvested by centrifugation at 12,000 *g* for 5 min at 4°C and washed twice with cold PBS. Lipids were extracted by the Bligh and Dyer procedure as described earlier (7). Solvent was evaporated under nitrogen and, unless otherwise indicated, lipids were saponified with 1 ml of 1 N NaOH at 70°C for 1 hr. After cooling, the nonsaponifiable lipids were extracted three times each with 3 ml of hexane, and the solvent was evaporated to dryness under a stream of nitrogen. The resulting residue was then analyzed by thin-layer chromatography (TLC), gas–liquid chromatography (GLC), and GLC–mass spectroscopy (MS). TLC was carried out on Gelman ITLC-SA polysilicic acid gel-impregnated sheets using hexane–diethylether–acetic acid 85:15:0.5 (v/v). This system separates sterols that differ in the number of methyl groups at C-4: cholesterol (4-desmethyl), R_f 0.16; 4α-methyl-cholest-7-en-3β-ol (4-monomethyl) R_f 0.23; lanosterol (4-dimethyl) R_f 0.30. It also separates the alcohols from their corresponding ketones: 4α-methyl-cholest-7-en-3-one, R_f 0.66. Gas–liquid chromatography was performed with a Hewlett Packard 5880A gas chromatograph using a 15 Sp-2550 capillary column at 250°C, and also as described earlier (7) except that 3% OV-17 on an 80/100 Supelcoport column was used and operated at 260°C. GLC–MS analysis for the identification of sterols was performed with a Finnigan 4021 GLC–MS spectrometer as described previously (14). NMR analyses were performed on a Nicolet 360WB (360 MHz) using $CDC1_3$ as solvent.

Growth on cholesterol precursors

Cells growing exponentially in the medium containing fetal calf serum were harvested by centrifugation at 3,000 *g* for 5 min at room temperature and washed once with RPMI 1640. The cells were then suspended in the growth medium containing 10% delipidated fetal calf serum instead of fetal calf serum. Aliquots of 10 ml each were added to culture flasks (50 ml, 25 cm², Falcon) containing 0.27 ml of 5% BSA in 0.14 M NaCl and 40 μl of ethanol containing the indicated sterol or sterol precursor as follows: Na acetate, 0.55 μmol; mevalonolactone, 0.18 μmol; cholest-7-en-3β-ol, 0.04 μmol; or cholesterol, 0.04 μmol. Flasks were incubated at 37°C in a humidified 5% CO_2 environment. At indicated times, 0.5-ml aliquots were removed for cell counts.

Conversion of precursor sterols to cholesterol

Exponentially growing cells were harvested and washed as described above. The cells were then suspended in RPMI 1640, and 0.5-ml aliquots containing $3–4 \times 10^6$ cells/ml were transferred to Petri dishes (Falcon, 100 × 15 mm) each containing 0.54 ml of 5% BSA in 0.14 M NaCl, 60 μl of ethanol containing 0 or 0.2 mg of the indicated sterol, and 20 ml of the medium containing 10% delipidated fetal calf serum. The plates were incubated for 48 hrs at 37°C in a humidified 5% CO_2 environment. The cells were then harvested by centrifugation at 4°C and washed twice with cold PBS. Lipids were extracted and quantitated by GLC using cholestane as internal standard.

[^{14}C]Acetate incorporation into sterols

[^{14}C]Acetate incorporation into sterols was carried out according to the method of Kandutsch and Chen (16); 2×10^6 U937 cells or U937-N cells were grown for 2 hr in delipidated serum as described above. [^{14}C]Acetate (2.5 μCi/μmol, 8 μmol) was added and cells were incubated for 2 hr at 37°C. Cells were harvested by centrifugation at 2,000 *g* for 10 min at 4°C and washed twice with PBS. The cells were digested by the addition of 1.5 ml of 0.1 N

NaOH and 150 μl was taken to determine the protein content. The rest of the sample was saponified overnight after addition of 1 ml of 20% KOH in 90% ethanol. Nonsaponifiable lipids were extracted with hexane and separated by thin-layer chromatography in hexane–ethyl acetate 85:15(v/v).

Enzyme assay conditions

The 3-ketosteroid reductase assay was similar to that previously described (17). In brief, each reaction mixture contained 0.05 mg of NADP, 0.05 mg of NADPH, 0.5 mg of DL-isocitrate, 0.125 units of isocitrate dehydrogenase, 5 mg of glucose, 10 units of glucose oxidase, 20 nmol of [^{3}H]-4α-methyl-7-en-3 one (5000 dpm/nmol), 0.32 mg of Triton WR-1339, 0.1 M potassium phosphate buffer, pH 7.4, and 1 mg of microsomal protein in a final volume of 0.2 ml. After a 5-min preincubation, the reaction was started by the addition of enzyme, and the reaction was stopped after 120 min at 37°C by the addition of 4 ml of $CHCl_3$–MEOH 2:1 (v/v); 0.8 ml of distilled H_2O was added and the chloroform layer was removed and evaporated to dryness. The steroids were separated by TLC and the ratio of radioactivity in the alcohol to the combined radioactivity in the alcohol and ketone represented the fraction of substrate converted to product. Under these conditions 3-ketosteroid reductase activity was linear with respect to time for up to 2 hr and with protein concentration up to 3 mg.

Protein was determined by the method of Lowry et al. (18).

RESULTS

Growth of U937

Previously, we had shown that U937 cells obtained from Dr. Hillel S. Koren would not grow in lipid-depleted serum unless cholesterol or a similar desmethyl sterol (desmosterol, 7-dehydrocholesterol) was present (7). Several approaches were taken to determine the portion of the cholesterol biosynthetic pathway that was defective in these cells. The cells were grown in the medium containing delipidated serum and supplemented with various intermediates in cholesterol biosynthesis **(Fig. 1).** Acetate, mevalonate, squalene, and 4,4-dimethyl-cholest-7-en-3-β-ol were incapable of supporting growth. Cholest-7-en-3-β-ol supported growth but less well than cholesterol. It can be concluded that the block was probably in one of the three enzymes involved in the demethylation of the two methyl groups at C-4 **(Fig. 2).**

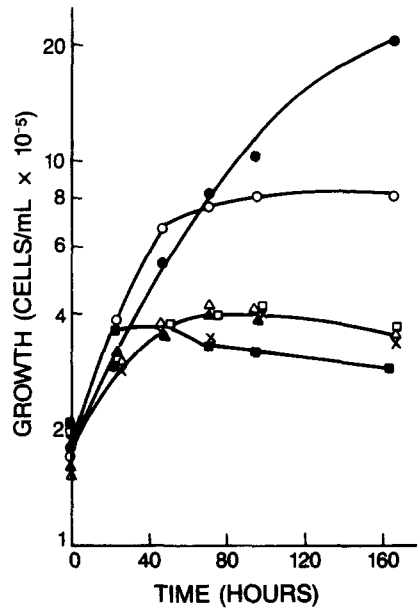

Fig. 1. Effects of cholesterol precursors on growth of U937. Cells growing exponentially in the medium containing fetal calf serum were harvested, washed, and suspended in the medium containing 10% delipidated fetal calf serum and the desired cholesterol precursor as indicated under Experimental Procedures. (△) Acetate; (▲) mevalonate; (□) squalene; (■) 4,4 dimethyl-cholest-7-en-3β-ol; (○) cholest-7-en-3β-ol; (●) cholesterol; (X) none.

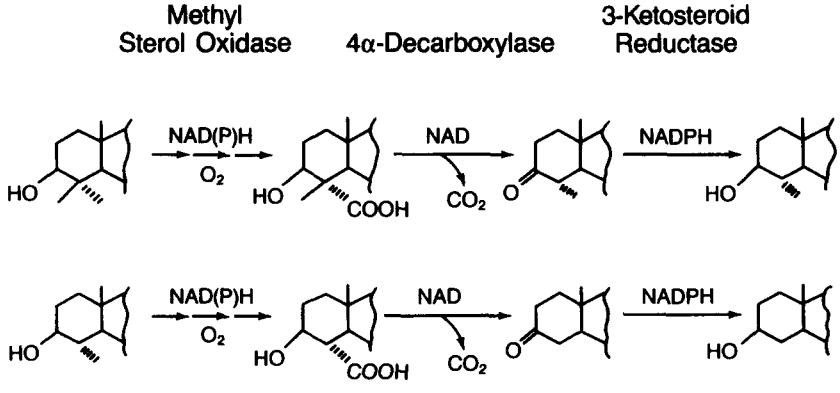

Fig. 2. Enzymes involved in the demethylation of the 4-gem dimethyl group of sterols.

Sterol composition of U937

When U937 cells were grown in medium containing fetal calf serum, the nonsaponifiable extract yielded two compounds; the major (90%) had the same retention time by GLC analysis as cholesterol, and the second (10%) had a relative retention time of 1.24 versus cholesterol. When U937 cells were grown for 48 hr on delipidated serum prior to extraction, the second had increased to almost 50% of the nonsaponifiable lipids and, in addition to cholesterol, four other sterols were found in small quantities **Fig. 3, Table 1).** The extract was further analyzed by a TLC system that separates sterols according to the number of methyl groups at C-4 (see Experimental Procedures). Regions of the TLC plate corresponding to the migration of desmethyl sterols, 4-mono-methyl-sterols, 4,4-dimethyl sterols, and 3-ketosteroids were extracted and analyzed by GLC and GLC–MS (Table 1). Analysis of the desmethyl region revealed one sterol, cholesterol. No sterols were found in the 4-mono-methyl region. The 4-dimethyl-region gave rise to three sterols (Fig. 3, peaks 3, 4, and 6, Table 1); these were identified as dihydrolanosterol, 4,4-dimethyl-cholest-8-en-3β-ol, and lanosterol, respectively. Two steroids were found in the 3-ketosteroid region of the TLC plate (Fig. 3, peaks 2 and 5, Table 1). That they were both ketones was shown by the characteristic absence of an M-18 peak in the mass spectra which corresponds to loss of water. The mass spectrum of peak 2, the major intermediate to build up, was similar to that of synthetic 4α-methyl-cholest-7-en-3-one [398 (M^+, 100%), 383 (M–CH_3, 47%), 285 (M–C_8H_{17}, 70%), 243 (M–$C_{11}H_{23}$, 41%)] and was designated as 4α-methyl-cholest-8-en-3-one. This structure was confirmed by NMR; 0.64 (s, 3H C-18-CH_3), 1.02 (d, 3H C-30-CH_3), 1.19 (s, 3H C-19-CH_3). The position of the C-18 at about 0.62 is characteristic of $\Delta^{8(9)}$ monoenes (14). Also, the absence of any signal between 5.2–5.4 shows that the double bond must be tetra-substituted. The second ketone, peak 5, had an additional degree of unsaturation which from MS fragmentation occurred in the side-chain and has been given the tentative assignment of 4α-methyl-cholest-8,24,dien-3-one. The build-up of 3-ketosteroids suggests that the defect is in the enzyme 3-ketosteroid reductase (see Fig. 2).

Conversion of precursor sterols to cholesterol

We previously reported that U937 cells convert desmosterol and 7-dehydrocholesterol to cholesterol (7) indicating that the steps from these sterols to cholesterol is intact. The data of **Table 2** reveal that the cells accumulate cholesterol when incubated with Δ^7-cholestenol, but do not do so when incubated with lanosterol. Thus, the cells incubated for 48 hr in the medium containing delipidated fetal calf serum and no sterols contained 5.0 ± 1.3 μg cholesterol per mg protein. The cells incubated with lanosterol contained a similar amount of cholesterol (4.4 ± 0.9 μg/mg protein). In contrast, cells incubated with choles-7-en-3-β-ol contained a significantly higher level of cholesterol (9.6 ± 0.5 μg cholesterol/mg protein). These observations further indicate that the defect in sterol synthesis lies at a step(s) in conversion of lanosterol to Δ^7-cholestenol.

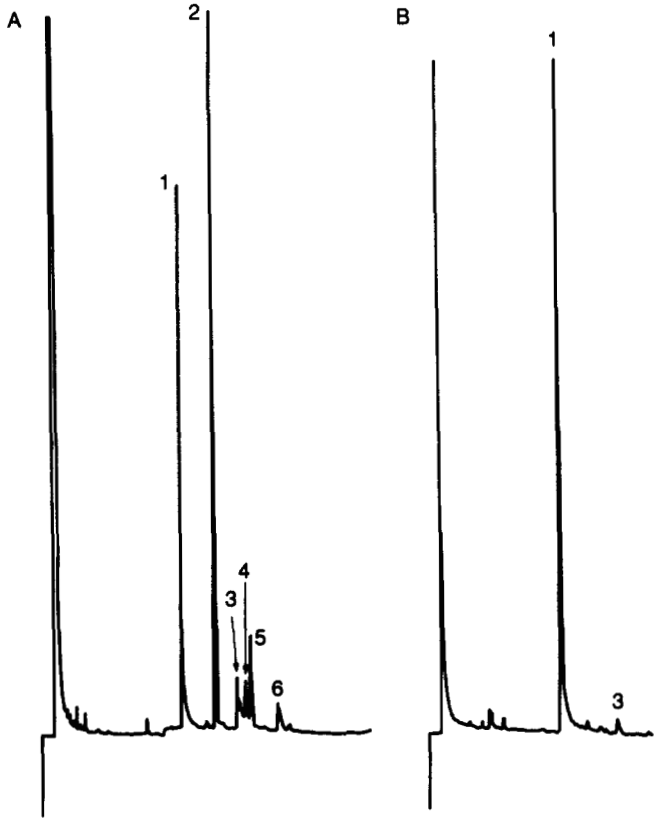

Fig. 3. Gas chromatogram of nonsaponifiable lipids isolated from U937 or U937-N cells grown in medium containing delipidated serum. Exponentially growing cells were harvested, washed, and incubated at a cell concentration of 0.35×10^6 cells/ml in the medium containing 10% delipidated fetal calf serum for 48 hr at 37°C. Nonsaponifiable lipids were isolated and chromatographed on an SP-2550 capillary column as described under Experimental Procedures. (A) U937; (B) U937-N. Retention times of various peaks are given in Table 1.

Requirement of variants of U937 for cholesterol

Fogelman et al. (19) have demonstrated that human monocytes are extremely active in the synthesis of cholesterol. This suggests that the U937 cells may be a mutant strain from the original cell line established by Sundström and Nilsson (3). Accordingly, a second isolate of U937 (designated U937-N) was obtained. The U937-N cells were capable of growing on delipidated serum. A lipid extract of U937-N cells grown in delipidated serum contained no peaks corresponding to 3-ketosteroid when analyzed by GLC, and cholesterol accounted for greater than 95% of the total cellular sterol (Fig. 3). In addition,

TABLE 1. Characterization of sterols isolated from U-937 cells incubated in medium containing delipidated serum

Peak	RRT[a] versus Cholesterol	% of Total	R_f by TLC	Mass Spectral Analysis	Structural Designation
1	1.0	33	4-Desmethyl	386(M+,100%) 371 (M-CH_3,39%) 368(M-H_2O,51%) 301(M-C_6H_{13},72%) 273(M-C_8H_{17},60%)	Cholesterol
2	1.24	48	Ketone	398(M+,100%) 383(M-CH_3,43%) 285(M-C_8H_{17},18%) 243(M-$C_{11}H_{23}$,26%)	4α-Methyl-cholest-8-en-3-one
3	1.39	3	4-Dimethyl	428(M+,14%) 413(M-CH_3,100%) 395(M-H_2O-CH_3,68%)	Dihydrolanosterol
4	1.45	3	4-Dimethyl	414(M+,100%) 399(M-CH_3,22%) 396(M-H_2O,11%) 381(M-H_2O-CH_3,23%)	4,4-Dimethyl-cholest-8-en-3β-ol
5	1.49	6	Ketone	396(M+,77%) 381(M-CH_3,100%) 243(M-$C_{11}H_{23}$,32%) 107(32%)	4α-Methyl-cholest-8,24-diene-3-one
6	1.68	3	4-Dimethyl	426(M+,34%) 411(M-CH_3,100%) 393(M-CH_3-H_2O,51%) 109(65%)	Lanosterol

[a]Relative retention time.

when U937-N cells grown in delipidated serum were pulsed for 2 hr with [^{14}C]acetate, 47% of the nonsaponifiable radioactivity comigrated on thin-layer chromatography with cholesterol. In contrast, only 2% of the label comigrated with cholesterol when U937 cells were pulsed with [^{14}C]acetate.

Activity of 3-ketosteroid reductase in U937 cells

Microsomes obtained from rat liver, U937 cells, and U937-N cells were assayed for 3-ketosteroid reductase activity **(Table 3).** The specific activity obtained in rat liver (545 pmol/min per mg) was similar to that previously obtained (17). The 3-ketoreductase activity in U-937N was only 1/25 of that in liver; this low activity in cell culture versus rat liver has also been observed in other enzymes of cholesterol metabolism, steroid-8-isomerase, and acyl-CoA cholesterol acyl transferase (Billheimer, J. T., unpublished data). The 3-ketoreductase activity in U-937 was undetectable under conditions employed and was at most 3% of that of U937-N.

DISCUSSION

Four approaches were taken to identify the lesion in cholesterol biosynthesis by U937 cells. These included: *a*) growth response to cholesterol precursors, *b*) identification of steroids that accumulate in cells incubated in the medium containing delipidated serum, *c*) ability of cells to convert sterol precursors to cholesterol, and *d*) assay of 3-ketosteroid reductase. The cells did not grow when supplemented with mevalonate, squalene, or 4,4-dimethyl-cholest-7-en-3β-ol (Fig. 1). They did grow on cholest-7-en-3β-ol, 7-dehydrocholesterol, or desmosterol (Fig. 1 and ref. 7). When incubated in the medium containing delipidated fetal calf serum, they accumulated 4α-methyl-8-en-3-one (Fig. 3 and Table 1) which was characterized by GLC, MS and NMR. The cells failed to produce cholesterol from lanosterol (Table 2), but converted cholest-7-en-3β-ol, 7-dehydrocholesterol, or desmosterol to cholesterol (Table 2 and ref. 7). Finally, cell-free extracts of U937 cells were found to be devoid of 3-ketosteroid reductase activity (Table 3). Taken together, these observations indicate that the metabolic defect lies in the inability of the cells to reduce the 4α-methyl ketone intermediate formed in the 4-demethylation of lanosterol. To our knowledge, this is the first demonstration of a cell that is deficient in 3-ketoreductase activity.

Experiments on the incorporation of radiolabeled acetate into nonsaponifiable lipids of U937 cells incubated in media containing delipidated serum showed that less than 2% of the radioactivity incorporated into nonsaponifiable lipids was found to comigrate with cholesterol. This sug-

TABLE 2. Effects of cholest-7-en-3β-ol or lanosterol on cholesterol content of U937 cells

Sterol in Medium	Cellular Sterol		
	Cholesterol	Cholest-7-en-3β-ol	Lanosterol
	μg/mg cell protein		
None	5.0 ± 1.3		
Cholest-7-en-3β-ol	9.6 ± 1.5	20.6 ± 1.0	
Lanosterol	4.4 ± 0.9		103.3 ± 1.3

Cells were incubated for 48 hr in the medium containing delipidated fetal calf serum and the desired sterol. Sterols were quantified as described under Experimental Procedures. Each value is mean ± range of two cultures.

TABLE 3. 3-Ketosteroid reductase activity in U937 cells

Enzyme	Activity
	pmol/min
Liver, 1 mg	545
U937-N, 1 mg	19
U937-N, 2 mg	32
U937, 1 mg	<1
U937, 2 mg	<1

About 1 × 10^9 cells were grown in FCS and switched to delipidated serum 20 hr prior to harvest. Cells were harvested by centrifugation, washed, and resuspended in 0.1 M phosphate buffer, pH 7.4, containing 2 mM glutathione (4 ml/10^9 cells). Cells were broken using a Tekmar Tissuemizer and microsomes were obtained by differential centrifugation.

gests that U937 is a stringent mutant. This is also suggested by the fact that cholesterol content of the cells grown for one generation (24 hr) in the medium containing delipidated serum is reduced by 60–70% (7). In contrast, in the CHO cell mutant No. 215 which is partially defective in methylsterol oxidase (cf. Fig. 2), the cholesterol content of the cells is reduced only 40% after 6 days (20). The cholesteryl ester content of U937 cells is very low, less than 1% of the cellular sterol (7). Nonetheless, the cells seem to contain enough excess cholesterol to support membrane synthesis for an initial growth period of 24 to 48 hr in the medium containing delipidated serum. A longer period of incubation in this medium results in loss of viability (data not shown).

Availability of U937 and U937-N should allow the use of molecular biology techniques to isolate and characterize the 3-ketosteroid reductase. This enzyme has been solubilized and partially purified; however, its lability has prevented purification to homogeneity by classical techniques (17). Furthermore, the defect we report here lies at a step committed to cholesterol synthesis, making U937 and U937-N an appropriate model for the studies of regulation of steps committed to cholesterol synthesis.

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REFERENCES

1. Zuckerman, S. H., and S. D. Douglas. 1979. Dynamics of the macrophage plasma membrane. *Annu. Rev. Microbiol.* **33:** 267–307.
2. Brown, M. S., and J. M. Goldstein. 1983. Lipoprotein metabolism in the macrophage: implications for cholesterol deposition in atherosclerosis. *Annu. Rev. Biochem.* **52:** 223–261.
3. Sundström, C., and K. Nilsson. 1976. Establishment and characterization of a human histiocytic lymphoma cell line (U-937). *Int. J. Cancer.* **17:** 565–577.
4. Lackman, L. B., J. O. Moore, and R. S. Metzar. 1978. Preparation and characterization of lymphocyte-activating factor (LAF) from acute monocytic and myelomonocytic leukemia cells. *Cell Immunol.* **41:** 199–206.
5. Koren, H. S., S. J. Anderson, and J. W. Larrick. 1979. In vitro activation of a human macrophage-like cell line. *Nature.* **279:** 328–331.
6. Minta, J. O., and L. Pambrun. 1985. In vitro induction of cytologic and functional differentiation of the immature human monocyte-like cell line U-937 with phorbol myristate acetate. *Am. J. Pathol.* **119:** 111–126.
7. Esfahani, M., L. Scerbo, and T. M. Devlin. 1984. A requirement for cholesterol and its structural features for a human macrophage-like cell line. *J. Cell Biochem.* **25:** 87–97.
8. Kandutsch, A. A., H. W. Chen, and H. Heiniger. 1978. Biological activity of some oxygenated sterols. *Science.* **201:** 498–501.
9. Gibbons, G. F., C. R. Pullinger, H. W. Chen, W. K. Cavenee, and A. A. Kandutsch. 1980. Regulation of cholesterol biosynthesis in cultured cells by probable natural precursor sterols. *J. Biol. Chem.* **255:** 395–400.
10. Gupta, A., R. C. Sexton, and H. Rudney. 1986. Modulation of regulatory oxysterol formation and low density lipoprotein suppression of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity by ketoconazole. *J. Biol. Chem.* **261:** 8348–8356.
11. Gautschi, F., and K. Bloch. 1958. Synthesis of isomeric 4,4-dimethylcholestenols and identification of lanosterol metabolite. *J. Biol. Chem.* **233:** 1343–1347.
12. Neiderhiser, D. H., and W. W. Wells. 1959. The structure of methostenol and its distribution in rat tissues. *Arch. Biochem. Biophys.* **81:** 300–308.
13. Menini, E., and J. K. Norymberski. 1962. The oxidation of sterols with *tert*-butyl chromate. *Biochem J.* **84:** 195–201.
14. Paik, Y-K., J. T. Billheimer, R. Magolda, and J. L. Gaylor. 1986. Microsomal enzymes of cholesterol biosynthesis from lanosterol: solubilization and purification of steroid 8-isomerase. *J. Biol. Chem.* **261:** 6470–6477.
15. Rothblat, G. H., L. Y. Arbogast, L. Quellette, and B. V. Howard. 1976. Preparation of delipidized serum protein for use in cell culture systems. *In vitro.* **12:** 554–557.
16. Kandutsch, A. A., and H. W. Chen. 1973. Inhibition of sterol synthesis in cultured mouse cells by 7α-hydroxycholesterol, 7β-hydroxycholesterol, and 7-ketocholesterol. *J. Biol. Chem.* **248:** 8408–8417.
17. Billheimer, J. T., M. Alcorn, and J. L. Gaylor. 1981. Solubilization and partial purification of a microsomal 3-ketosteroid reductase of cholesterol biosynthesis. *Arch. Biochem. Biophys.* **211:** 430–438.
18. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
19. Fogelman, A. M., J. Seager, P. A. Edwards, M. Hokom, and G. Popják. 1977. Cholesterol biosynthesis in human lymphocytes, monocytes, and granulocytes. *Biochem. Biophys. Res. Commun.* **76:** 167–173.
20. Chang, T. Y., C. Telakowski, W. Heuvel, A. W. Alberts, and P. R. Vagelos. 1977. Isolation and partial characterization of a cholesterol-requiring mutant of Chinese hamster ovary cells. *Proc. Natl. Acad. Sci. USA.* **74:** 832–836.