# Biological Activity of Some Oxygenated Sterols

Andrew A. Kandutsch, Harry W. Chen, Hans-Jörg Heiniger

*Summary.* A group of oxygenated sterols has been identified as potent and specific inhibitors of sterol biosynthesis. The ability of these compounds to inhibit sterol synthesis in cultured cells and the ineffectiveness of cholesterol under the same conditions suggest that feedback regulation of sterol biosynthesis may be brought about by an oxygenated sterol rather than by cholesterol. The nature of the regulatory sterol may vary in different cells with their specific requirements for cholesterol as a structural component or as a precursor of other steroid products. The use of oxygenated sterols to block sterol synthesis in cultured cells provides new information regarding the role of sterol in cell membrane structure and function. For example, de novo sterol synthesis is required for DNA synthesis and cell division by some cultured cells. Studies with cultured cells, and with rats and mice in vivo, suggest that oxygenated sterols could be of value in the treatment of several important human diseases.

The recent observation that certain oxygenated derivatives of cholesterol are potent inhibitors of sterol biosynthesis in mammalian cells is of major interest for three reasons. (i) It brings into question the supposition that cholesterol regulates its own synthesis. (ii) By providing a means through which cholesterol biosynthesis can be specifically inhibited, it permits investigations into the role of sterol in various cell functions. (iii) Potential pharmacological uses of the compounds are apparent. Although much remains to be learned, especially about mechanisms, it may be useful at this time to point out some of the implications of information that have been obtained so far.

## Regulation of Cholesterol Synthesis

The belief that regulation of sterol biosynthesis involves feedback control by cholesterol is founded principally on the observation that dietary cholesterol suppresses hepatic cholesterol synthesis at the level of the reaction catalyzed by the regulatory enzyme in the pathway, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (*1*). However, this effect of dietary cholesterol is not conclusive evidence that it acts directly as a regulatory molecule. Indeed, when they are fed with the diet, some sterols that are produced metabolically from cholesterol by irreversible reactions (such as bile acids, cholest-4-en-3-one, 5$\alpha$-cholestan-3$\beta$-ol) inhibit sterol synthesis as effectively as cholesterol does (*2*). Inhibition of hepatic cholesterol synthesis by such a wide variety of structurally and metabolically dissimilar sterols points up the complexity of the system in vivo. Cholesterol is taken into the liver from the blood; excess amounts are esterified and stored, and the remainder is secreted into the blood as a lipoprotein component and into the intestine either unchanged or after conversion into bile acids. Most of the cholesterol secreted into the intestine is resorbed into the blood stream. Therefore, any factor that alters any one of these processes may affect concentrations of hepatic cholesterol, cholesterol esters, bile acids, and intermediates between cholesterol and bile acids. In addition, many hormones including insulin, glucagon, testosterone, glucocorticoids, thyroxine, and epinephrine can influence the level of HMG-CoA reductase activity and the rate of sterol synthesis in liver (*1*). Thus the suppression of hepatic cholesterol synthesis by dietary sterols could be a secondary result of effects on cholesterol catabolism or upon a function of some organ other than the liver.

Only hepatic sterol synthesis is rapidly suppressed by dietary cholesterol. Even when the dietary sterol has elevated the concentration of cholesterol in the general circulation, sterol synthesis in the epidermis (*3*) and intestine (*4, 5*) and in leukocytes, leukemic cells (*6*), and hepatomas (*7*) is little affected although their rates of synthesis may be as high as that in liver. Sterol synthesis in these extrahepatic cells is, therefore, largely independent of the amount of circulating cholesterol. Because the central nervous system is separated by the blood-brain barrier from cholesterol in the serum lipoproteins, dramatic changes in the rate of sterol synthesis in developing brain are of particular interest. Sterol synthesis in mouse brain is high at birth and declines thereafter, except for a burst of synthesis when the animals are between 5 and 20 days of age which is associated with the development of myelin. When the animals are 40 days of age, brain growth and sterol synthesis have essentially ceased and, since sterol in the brain is isolated from that in the circulation, there is little exchange and the turnover of cholesterol in mature brain is extremely slow (*8*). The fact that there is very little turnover (or synthesis) of sterol in mature brain indicates that appreciable turnover of cholesterol is not an invariant and vital requirement for nondividing cells.

Evidence that cholesterol biosynthesis may be regulated by a sterol (or sterols) other than cholesterol came from investigations with cultured cells. The earliest studies indicated that sterol synthesis in fibroblast cultures was suppressed when serum, serum lipids, or any one of several sterols (cholesterol, desmosterol, lathosterol, 7-dehydrocholesterol, 5$\alpha$-cholestan-3$\beta$-ol, cholest-4-en-3-one, and lanosterol) was added to the culture medium (*9*). Inhibition of sterol synthesis in cell cultures treated with cholesterol preparations was confirmed in other laboratories (*10, 11*), and the results obtained with this experimental system appeared to be analogous to the suppression of hepatic cholesterogenesis by dietary cholesterol. However, studies in our laboratory and by others showed that trace amounts of contaminating sterols could account for all of the inhibitory activity of various cholesterol preparations in cell cultures (*12*). Furthermore, purified cholesterol was not inhibitory when it was incubated with cultures of mouse or rat liver cells (*13*), mouse lymphocytes (*14*), human fibroblasts (*13*), or Chinese hamster lung cells (*15*). Pure cholesterol at a high concentration ($10^{-5}M$) was weakly inhibitory when it

The authors are members of the Research Staff of the Jackson Laboratory, Bar Harbor, Maine 04609.



0036-8075/78/0811-0498$01.00/0 

was incubated for long periods of time (12 to 72 hours) with highly sensitive L-cell (mouse fibroblast) cultures in the absence of an antioxidant, but effected no inhibition when vitamin E was present in the medium (*16*). We also demonstrated that sterols, other than cholesterol, with a single hydroxyl or ketone function (including those reported to be inhibitory) had no effect on sterol synthesis in cultures of fibroblasts or mouse liver cells after they had been extensively purified (*12, 17*).

Although the inhibitory contaminants in impure cholesterol preparations have not been conclusively identified, a number of oxygenated sterols have been found to inhibit sterol synthesis when added to cultures at concentrations in the range of $10^{-9}$ to $10^{-6}M$ (*12, 17*). These inhibitors can all be named as derivatives of 5$\alpha$-cholestan-3$\beta$-ol. They have one hydroxyl or ketone function at position 3 and another at one of the numbered positions of the structure shown in Fig. 1. The presence or absence of a double bond at position 5 does not appreciably affect the inhibitory potencies of sterols tested in this respect, but the complete side chain appears to be required for full activity. The majority of the sterols with proven inhibitory activity can therefore be derived by the oxidation of cholesterol (cholest-5-en-3$\beta$-ol).

Some of the most potent inhibitors (such as 7-ketocholesterol, 25-hydroxycholesterol) are readily produced by autoxidation of cholesterol in air at room temperature, and the rate of oxidation is accelerated by light and by $Cu^{2+}$ or $Fe^{2+}$ ions (*18*). Autoxidation products could, therefore, account for inhibitory activity apparently associated with cholesterol if the sterol preparation was not extensively purified shortly before it was used. Cholesterol dispersions in aqueous solutions also undergo autoxidation in the presence of atmospheric oxygen (*18*). Evidence has been presented (*12, 19*) that autoxidation of cholesterol occurred when it was incubated in cell culture media in an atmosphere of air in the presence and absence of cells. Oxygenated sterols generated in this way probably account for the weak inhibitory activity frequently associated with cholesterol when it is incubated for prolonged periods of time with cell cultures in the absence of an antioxidant.

Some of the oxygenated sterols which are known to be inhibitory are normal metabolites in cells and may function there as regulators. Hydroxylation of cholesterol to give 7$\alpha$-hydroxycholesterol is the first step in bile acid production. Similarly, hydroxylation to give

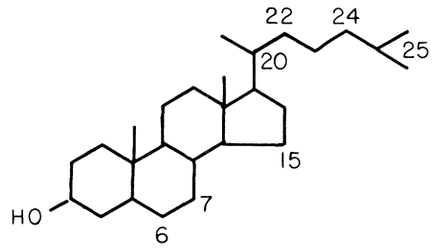


Fig. 1. 5$\alpha$-Cholestan-3$\beta$-ol. Introduction of a ketone or hydroxyl function at any one of the numbered positions produces a potent inhibitor of sterol synthesis.

20$\alpha$-hydroxycholesterol occurs during the production of steroid hormones from cholesterol. Recent reports (*20*) demonstrating the enzymatic production of (24*S*)-24-hydroxycholesterol (cerebrosterol) in brain are noteworthy in this respect because this inhibitory sterol could be involved in determining the marked dramatic changes in sterol production that accompany development of the central nervous system. Whereas oxygenated products of cholesterol metabolism may play a regulatory role in the organs mentioned above, most of the cells of the organism do not convert cholesterol into any of the known inhibitory oxygenated sterols. It is possible that in these cells a dihydroxy precursor of cholesterol functions as a regulator since sterols with a 14-hydroxymethyl group or with a 15-hydroxyl function have been shown to inhibit sterol synthesis in cultured cells (*21*).

Obvious advantages are associated with the control of cholesterol synthesis by different sterols in different organs. In liver and in endocrine glands, the supply of sterol must conform with the need of the organism for secretory products (lipoproteins, bile acids, and steroid hormones); in brain it must conform with cell division during development and with the myelination process; in most other cells it must conform with cell division and possibly, under some conditions, with differentiation and with repair and maintenance of cell membranes. The system that regulates the activity of HMG-CoA reductase is sensitive and able to bring about rapid changes in the rate of sterol synthesis. It seems adequate, therefore, to meet discontinuous and quantitatively different requirements for cholesterol by various cells of the organism.

The oxygenated sterols inhibit cellular sterol synthesis by depressing the activity of HMG-CoA reductase. In the presence of relatively high concentrations of the most powerful inhibitors, the enzyme activity in fibroblasts and in rat hepatoma cultures declines with a half-life of approximately 1 hour. The half-life of the enzyme activity found under these conditions is not inconsistent with estimates ranging from 1 to 4 hours for the half-life of the hepatic enzyme in vivo—values obtained through other procedures (*1*). The mechanism through which HMG-CoA reductase activity is suppressed by the oxygenated sterols is not yet clear. The sterols do not affect the activity of the enzyme when they are mixed directly with microsomal enzyme preparations (*12*). Thus, it seems that they must either suppress the synthesis of the enzyme, increase the rate of its degradation, or inactivate it by some indirect mechanism. In regard to this question, it is useful to consider what is known of mechanisms by which other regulatory fluctuations in HMG-CoA reductase are accomplished. A marked diurnal cycle in hepatic HMG-CoA reductase in vivo is brought about by alterations in the rate of enzyme synthesis. Induction of HMG-CoA reductase activity by injecting Triton WR1339 (a detergent) or by feeding cholestyramine (a resin that increases the excretion of bile acids from the intestine) also appears to involve an increase in the rate of enzyme synthesis (*1, 22*). The synthesis of HMG-CoA reductase is suppressed when cholesterol is added to the diet (*22*). Thus, it is reasonable to postulate repression of the synthesis of HMG-CoA reductase by the oxygenated sterols as an explanation for their effect in cultured cells and on liver and intestine in vivo. In addition, dietary cholesterol appears to cause inactivation of the enzyme before it represses its synthesis, suggesting that it has two effects in liver (*23*). Bierne *et al.* (*24*) and Bell *et al.* (*11*) have presented data that, they suggest, indicate that the initial effect of 25-hydroxycholesterol in rat hepatoma cell cultures is to inactivate HMG-CoA reductase or to increase the rate of its degradation. Whether the oxygenated sterols repress the synthesis of the enzyme or inactivate it by some indirect mechanism, it is likely that the mechanism of action involves binding of the inhibitor to specific sites on some macromolecule or subcellular structure. Consistent with this expectation, binding of 25-hydroxycholesterol to a cytosolic protein (or proteins) different from the protein (or proteins) that binds cholesterol has been demonstrated (*25*).

The oxygenated sterols suppress sterol synthesis in vivo as well as in cell cultures, although their effects in rats and mice are different from those of cholesterol. Dietary 7-ketocholesterol and 25-hydroxycholesterol suppressed sterol synthesis in the intestines but not in the

livers of mice (*5*), whereas in rats dietary 7-ketocholesterol caused transient inhibition of hepatic sterol synthesis (*26*). Apparently, the inhibitory effects of the oxygenated sterols tested in vivo are limited by their rapid metabolism and by their ability to induce enzyme systems that result in their inactivation (*26*). In addition to anticipated effects of oxygenated sterols on sterol synthesis in vivo, dietary 3$\beta$-hydroxy-5$\alpha$-cholest-8(14)-en-15-one produced a marked depression in the level of blood cholesterol in rats (*27*), and all of the inhibitors tested so far caused a dramatic suppression of appetite when they were fed with the diet to mice and rats (*5, 26, 27*). Appetite suppression was not due to an unpalatable flavor of the sterols since mice did not exhibit an aversion to diet containing 25-hydroxycholesterol, 7-ketocholesterol, or 20$\alpha$-hydroxycholesterol when given the choice of an alternative diet containing cholesterol. It has not been established whether the effects of the oxygenated sterols upon appetite and blood cholesterol levels are directly related to the inhibition of sterol synthesis.

### Consequences of Blocked Sterol Synthesis

Apparently all mammalian cell cultures synthesize sterols when grown in medium containing serum from which the lipids have been removed or in medium from which serum is omitted. Many cultures also synthesize sterol at appreciable rates when complete serum is present in the medium (*19, 28*). However, removal of the serum lipids generally stimulates the rate of sterol synthesis. It is not clear to what extent the suppressive effect of serum lipids on sterol synthesis is due to oxygenated sterols, either present at the time of their use, or generated from cholesterol during incubation of the serum, or serum lipids, with the culture. 7$\beta$-Hydroxycholesterol was isolated from horse serum in 1940 (*29*) and was found to be present in several samples of fetal calf serum used in our laboratory for culturing cells. The amount of 7$\beta$-hydroxycholesterol increased when the serum was incubated at 37°C with the cultures (*19*).

When an oxygenated sterol (such as, 25-hydroxycholesterol or 7-ketocholesterol) is added to the serum-free medium of L-cell cultures, HMG-CoA reductase activity and the rate of sterol synthesis decline and are nearly completely suppressed within a period of hours. Concomitant with (or immediately following) the suppression of HMG-CoA reductase the concentration of cellular sterol and the rate of DNA synthesis also decline. At 24 to 36 hours after addition of the inhibitor, growth of the culture has essentially stopped, and after 48 hours the cells become rounded and detached from the surface of the flask (*12, 16, 30*). Apparently the oxygenated sterols block cell division in the $G_1$ phase of the cell cycle (*13, 31*). Roughly coinciding with the cessation of growth, the concentrations of sterol in the whole cells and in the plasma membrane reach a minimum value approximating one-half of the initial level. This diminished concentration of sterol is maintained thereafter as long as enough cells for testing survive in the culture flask (*16*). No significant changes in the concentrations of phospholipids occur in the blocked cells, and thus the molar ratio of sterol to phospholipids is diminished from approximately 0.4 to 0.2. Because the viscosity of the lipid core of the plasma membrane is a function of the concentration of sterol, a decrease in the ratio of cholesterol to phospholipids indicates an increase in membrane fluidity, provided that compensatory changes in other membrane components do not occur (*32*). The increased fluidity of the plasma membrane in sterol-deficient L cells alters several of its functions. There is an increase in the rate of rubidium transport by the $Na^+$, $K^+$-activated adenosine triphosphatase system, an increase in the passive efflux of rubidium from the cells (*33*), as well as increased membrane fragility (*16*). The sterol-depleted cells are unable to take up soluble horseradish peroxidase by pinocytosis, indicating that vesicle formation and internalization of the plasma membrane is dependent on the maintenance of a suitable level of rigidity in the lipid core of the membrane (*34*).

It is not clear to what extent changes in membrane function accompanying the blockage of sterol synthesis are related to the cessation of DNA synthesis and cell division. Obviously, cell replication requires the production of new membrane, and cholesterol is required for this. It may be that the failure to produce cholesterol for membrane formation during an early stage of the cell cycle prevents further steps leading to mitosis. The requirement for additional cholesterol during a particular stage of cell division is illustrated by the results of studies with lectin-stimulated lymphocytes and leukemic cells (*13, 35*). Soon after the addition of phytohemagglutinin to a normal lymphocyte culture, a cycle of sterol synthesis commences and reaches a peak after about 24 hours. Synthesis of DNA begins after about 24 hours and reaches its peak at 48 hours. If the synthesis of cholesterol is suppressed by an oxygenated sterol during the first 24 hours of culture, the subsequent synthesis of DNA and cell division are also suppressed. After the synthesis of cholesterol has reached its peak, the addition of an oxygenated sterol does not affect the subsequent phases of the cell cycle. A similar but somewhat faster sequence of events occurred in leukemic cells that had been stimulated with a relatively low concentration of lectin.

It is important that the sterol requirements of L-cell cultures incubated with an oxygenated sterol can be met by exogenously supplied cholesterol, desmosterol, or mevalonate (the product of the reaction catalyzed by HMG-CoA reductase). Growth rates, concentrations of cellular and membrane sterol, rates of $Rb^+$ transport and endocytosis can be maintained for several generations in L cells that are prevented from synthesizing sterol provided that desmosterol, cholesterol, or mevalonate is present in the medium (*16, 30, 33, 34*). In contrast, a cycle of de novo sterol synthesis was a prerequisite for DNA synthesis in primary cultures of mouse or human lymphocytes, even though the cells were cultured in medium containing lipoprotein cholesterol or cholesterol dispersed with albumin (*13, 35*).

In trying to understand an apparent requirement for endogenous sterol synthesis by dividing lymphocytes and the lack of such a strict requirement by dividing L cells, it may be important to consider the origins of the two kinds of cell culture. Probably, established lines of cultured cells grown in medium containing serum have frequently (if not continuously) been exposed to products of cholesterol autoxidation (*19*). Cell lines established in the presence of these inhibitory compounds may have been selected for an ability to grow and divide without synthesizing cholesterol. One possible mode of adaptation would be to increase their ability to take up cholesterol from the medium.

Inherent in this analysis is an assumption that cells normally (that is, in vivo) synthesize sterol needed for cell division even though cholesterol may be present in extracellular fluids. Several kinds of evidence support this assumption. The most direct evidence—that obtained in studies with lectin-stimulated primary cultures of lymphocytes—has already been described. Perhaps of most interest in this regard are reports showing that cells proliferating in vivo synthesize cholesterol at high rates that are essentially

independent of dietary cholesterol or of blood levels of the sterol. Proliferating tissues and cells with high rates of sterol synthesis include intestinal mucosa, epidermis, spontaneous—or well differentiated—transplantable tumors (*3, 4, 6-8, 36*), and, as was previously mentioned, developing brain. However, nondividing muscle cells do not synthesize sterol at appreciable rates, and mature brain synthesizes little sterol even though it is isolated from blood cholesterol. The high rates of sterol synthesis in proliferating cells in vivo, largely independent of circulating levels of cholesterol, suggest that de novo sterol synthesis is a requirement for division of these cells under physiological conditions, as it is in lymphocyte cultures in vitro.

## Pharmacological Implications

Although pharmacological control over cholesterol biosynthesis has long been sought as a means for regulating the amount of cholesterol in the blood and for the prevention and treatment of atherosclerosis (*37*), specific inhibitors of sterol synthesis have not, heretofore, been available. Several complications limit the use for this purpose of the inhibitory sterols that have been studied so far. Those inhibitors that have been most thoroughly tested (7-ketocholesterol and 25-hydroxycholesterol) appear to be metabolized so rapidly in the liver that inhibition of hepatic and intestinal cholesterol synthesis is transient and their effects on blood levels of cholesterol are minimal (*26*). It should be possible, through further study, to diminish this obstacle by altering the structures of the inhibitory molecules so as to reduce their metabolism, by finding a more effective way to administer them, or by a combination of these. The observation that 3$\beta$-hydroxy-5$\alpha$-cholest-8(14)-en-15-one, administered orally or subcutaneously, lowered blood cholesterol, and that the palmitate and hemisuccinate esters of this compound and 14$\alpha$-ethyl-5$\alpha$-cholest-7-en-3$\beta$,15$\alpha$-diol were also active in this regard when injected subcutaneously into rats, offers hope that this end can be attained (*27, 38*). However, studies with oxygenated sterols have brought to light some previously unforeseen consequences of blocked sterol synthesis. If sterol synthesis is required for cell division in vivo, as appears to be the case, then administration of a potent inhibitor of sterol synthesis may block cell division in intestine, bone marrow, lymphoid tissues, and epidermis to the detriment of the organism. These effects, if significant in vivo, could limit the therapeutic use of the sterols to reduce serum cholesterol. In contrast, the same effects of the oxygenated sterols on cell division might be useful in the control of abnormal rates of cell division, such as those occurring in cancers. It is also possible that the dramatic effect of the oxygenated sterols on appetite can be exploited to reveal new ways for regulating food consumption. Much remains to be learned before further speculation regarding these questions is profitable.

### References and Notes


1. For a recent review, see V. W. Rodwell, J. L. Nordstrom, J. J. Mitschelen, *Adv. Lipid Res.* **14**, 1 (1976).
2. G. M. Tomkins, H. Shepard, I. L. Chaikoff, *J. Biol. Chem.* **203**, 781 (1953); W. T. Beher, G. D. Baker, W. L. Anthony, *Proc. Soc. Exp. Biol. Med.* **109**, 863 (1962); A. A. Kandutsch and R. M. Packie, *Arch. Biochem. Biophys.* **140**, 122 (1970); P. Back, B. Hamprecht, F. Lynen, *ibid.* **133**, 11 (1969).
3. N. Nicolaides and S. Rothman, *J. Invest. Dermatol.* **24**, 125 (1955); S. C. Brooks, V. C. Godfroi, W. L. Simpson, *ibid.* **40**, 305 (1963); J. L. Gaylor, *Arch. Biochem. Biophys.* **101**, 108 (1963); P. G. Brannan, J. L. Goldstein, M. S. Brown, *J. Lipid Res.* **16**, 7 (1975).
4. J. M. Dietschy and M. D. Siperstein, *J. Clin. Invest.* **44**, 1311 (1965); *J. Lipid Res.* **8**, 97 (1967); J. L. Merchant and R. A. Heller, *ibid.* **18**, 722 (1977); H. Muroya, S. Sodhi, R. G. Gould, *ibid.*, p. 301; S. Shefer, S. Hauser, V. Lapar, E. H. Mosbach, *ibid.* **14**, 400 (1973).
5. A. A. Kandutsch, H.-J. Heiniger, H. W. Chen, *Biochim. Biophys. Acta* **486**, 260 (1977).
6. H. W. Chen, A. A. Kandutsch, H.-J. Heiniger, H. Meier, *Cancer Res.* **33**, 2774 (1973); H. W. Chen and H.-J. Heiniger, *ibid.* **34**, 1304 (1974); H.-J. Heiniger, H. W. Chen, O. L. Applegate, Jr., L. P. Schacter, B. Z. Schacter, P. N. Anderson, *J. Mol. Med.* **1**, 109 (1976); N. L. Young and V. W. Rodwell, *J. Lipid Res.* **18**, 572 (1977).
7. J. D. McGarry and D. W. Foster, *J. Biol. Chem.* **244**, 4251 (1969); A. A. Kandutsch and R. L. Hancock, *Cancer Res.* **31**, 1396 (1971); M. D. Siperstein, *Can. Cancer Conf.* **7**, 152 (1967).
8. A. N. Davidson, *Adv. Lipid Res.* **3**, 171 (1965); A. A. Kandutsch and S. E. Saucier, *Arch. Biochem. Biophys.* **135**, 201 (1969).
9. M. J. Bailey, *Biochim. Biophys. Acta* **125**, 226 (1966); G. H. Rothblat and M. K. Buchko, *J. Lipid Res.* **12**, 647 (1971); J. Avigan, C. D. Williams, J. P. Blass, *Biochim. Biophys. Acta* **218**, 381 (1970).
10. M. S. Brown and J. L. Goldstein, *J. Biol. Chem.* **249**, 7306 (1974).
11. J. J. Bell, T. E. Sargeant, J. A. Watson, *ibid.* **251**, 1745 (1976).
12. A. A. Kandutsch and H. W. Chen, *ibid.* **248**, 8408 (1973); *ibid.* **249**, 6057 (1974); *J. Cell. Physiol.* **85**, 415 (1975); G. J. Schroepfer, Jr., E. J. Parish, A. A. Kandutsch, *J. Am. Chem. Soc.* **99**, 5494 (1977); R. C. Lin and P. J. Snodgrass, *FEBS Lett.* **83**, 89 (1977).
13. H. W. Chen, H.-J. Heiniger, A. A. Kandutsch, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1950 (1975).
14. J. L. Breslow, D. A. Lathrop, D. R. Spaulding, A. A. Kandutsch, *Biochim. Biophys. Acta* **298**, 10 (1975).
15. H. W. Chen, W. C. Cavenee, A. A. Kandutsch, *J. Biol. Chem.*, in press.
16. A. A. Kandutsch and H. W. Chen, *ibid.* **252**, 409 (1977).
17. H. W. Chen and A. A. Kandutsch, in *Atherosclerosis Drug Discovery*, C. E. Day, Ed. (Plenum, New York, 1976), p. 405.
18. L. L. Smith, W. S. Matthews, J. C. Price, R. C. Bachman, B. Reynolds, *J. Chromatog.* **27**, 187 (1967); J. E. VanLier and L. L. Smith, *J. Org. Chem.* **35**, 2627 (1970); L. L. Smith, J. I. Teng, M. J. Kulig, F. L. Hill, *ibid.* **38**, 1763 (1973); J. E. VanLier, A. L. DaCosta, L. L. Smith, *Chem. Phys. Lipids* **14**, 327 (1975); J. I. Teng, M. J. Kulig, L. L. Smith, J. E. VanLier, *J. Org. Chem.* **38**, 119 (1973); N. D. Weiner, P. Noomnont, A. Felmeister, *J. Lipid Res.* **13**, 252 (1972).
19. H. W. Chen and A. A. Kandutsch, in *The Nutritional Requirements of Vertebrate Cells In Vitro*, R. G. Ham and C. Waymouth, Eds. (Univ. of Cambridge Press, New York, in press).
20. A. K. Dhar, J. I. Teng, L. L. Smith, *J. Neurochem.* **21**, 51 (1973); Y. Y. Lin and L. L. Smith, *ibid.* **25**, 659 (1975).
21. G. J. Schroepfer, Jr., E. J. Parish, H. W. Chen, A. A. Kandutsch, *J. Biol. Chem.* **252**, 8975 (1977); S. Huntoon, B. Fourcans, B. N. Lutsky, E. J. Parish, H. Emery, F. F. Knapp, Jr., G. J. Schroepfer, Jr., *ibid.* **253**, 775 (1978); G. J. Schroepfer, Jr., R. A. Pascal, Jr., R. Shaw, A. A. Kandutsch, *Biochem. Biophys. Res. Commun.* in press.
22. M. Higgins, T. Kawachi, H. Rudney, *Biochem. Biophys. Res. Commun.* **45**, 138 (1971); M. Higgins and H. Rudney, *Arch. Biochem. Biophys.* **163**, 271 (1974); A. A. Kandutsch and S. E. Saucier, *J. Biol. Chem.* **244**, 2299 (1969).
23. M. Higgins and H. Rudney, *Nature (London) New Biol.* **246**, 60 (1973).
24. O. R. Bierne, R. Heller, J. A. Watson, *J. Biol. Chem.* **252**, 950 (1977).
25. A. A. Kandutsch, H. W. Chen, E. P. Shown, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 2500 (1977).
26. S. A. Erickson, A. D. Cooper, S. M. Matsui, R. G. Gould, *J. Biol. Chem.* **252**, 5186 (1977).
27. A. Kisic, A. S. Taylor, J. S. Chamberlain, E. J. Parish, G. J. Schroepfer, Jr., *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **37**, 1663 (1978); G. J. Schroepfer, Jr., D. Monger, A. S. Taylor, J. S. Chamberlain, E. J. Parish, A. Kisic, A. A. Kandutsch, *Biochem. Biophys. Res. Commun.* **78**, 1227 (1977); D. L. Raulson *et al.*, *ibid.* **71**, 984 (1976).
28. J. T. Cooper and S. Goldstein, *In Vitro* **13**, 473 (1977).
29. O. Wintersteiner and J. A. Ritzmann, *J. Biol. Chem.* **136**, 697 (1940).
30. H. W. Chen, A. A. Kandutsch, C. Waymouth, *Nature (London)* **251**, 419 (1974).
31. R. Cornell, G. L. Grove, G. H. Rothblat, A. F. Horwitz, *Exp. Cell. Res.* **109**, 299 (1977).
32. R. A. Demel and B. DeKruyff, *Biochim. Biophys. Acta* **457**, 109 (1976).
33. H. W. Chen, H.-J. Heiniger, A. A. Kandutsch, *J. Biol. Chem.* **253**, 3180 (1978).
34. H.-J. Heiniger, A. A. Kandutsch, H. W. Chen, *Nature (London)* **263**, 515 (1976).
35. H. W. Chen, H.-J. Heiniger, A. A. Kandutsch, *Exp. Cell Res.* **109**, 299 (1977); H. P. M. Pratt, P. A. Fitzgerald, A. Saxon, *Cell. Immunol.* **32**, 160 (1977).
36. H. W. Chen, A. A. Kandutsch, H.-J. Heiniger, *Prog. Exp. Tumor Res.* **22**, 275 (1978).
37. G. Gould, in *Cholesterol Metabolism and Lipolytic Enzymes*, J. Polonovski, Ed. (Masson, New York, 1977), pp. 14-38.
38. A. Kisic, D. Monger, E. J. Parish, S. Satterfield, D. L. Raulston, G. J. Schroepfer, Jr., *Artery* **3**, 421 (1977); A. Kisic, A. S. Taylor, J. S. Chamberlain, E. J. Parish, G. J. Schroepfer, Jr., personal communication.


39. Supported by NIH grants CA-02758, CA-19305, and GM-22900. We thank Dr. M. J. James, Dr. D. L. Coleman, and E. P. Shown for constructive criticism of the manuscript.