Receptor-Mediated Control of Cholesterol Metabolism

Study of human mutants has disclosed how cells regulate a substance that is both vital and lethal.

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Cholesterol, a major component of all mammalian plasma membranes, is vital to cell growth and survival (*1, 2*); yet, excessive amounts of this sterol can also be lethal, as is evidenced by the cholesterol deposition in arterial cells that potentiates the development of atherosclerosis (*3*). Thus, mammalian cells are faced with the dual problem of providing sufficient cholesterol for membrane growth and replenishment and, at the same time, of avoiding excessive accumulation of this insoluble substance.

In this article we discuss a mechanism by which certain mammalian cells, such as cultured human fibroblasts, utilize a specific cell surface receptor to accomplish their dual task. This receptor, designated the low density lipoprotein (LDL) receptor, binds the major cholesterol-carrying lipoprotein of plasma and thereby regulates the rate at which this lipoprotein transfers its cholesterol into the cell. The LDL receptor itself is under feedback regulation so that its activity (and hence the amount of cholesterol that enters the cell) is inversely proportional to the cellular content of cholesterol. Thus, cultured fibroblasts obtain cholesterol by increasing the number of receptor molecules, and conversely they protect themselves against an overaccumulation of the sterol by suppressing the synthesis of LDL receptors.

Interaction of LDL with Cells

In man, LDL is the lipoprotein that carries most of the cholesterol in plasma. About three-fourths of the cholesterol in LDL is esterified to long chain fatty acids and these cholesteryl esters are believed to be located in an apolar core of neutral lipid that is surrounded by phospholipid, unesterified cholesterol, and a protein called apoprotein B. Of the various cholesteryl esters found in human LDL, cholesteryl linoleate (18 carbon atoms, and two double bonds; the notation is C 18 : 2) accounts for the largest fraction (about 50 percent) (*4*). In studying the metabolism of this complex molecule by human fibroblasts, we have utilized LDL labeled either in its protein component with ^{125}I (which attaches mainly to tyrosine residues) or in its cholesteryl ester component with [^{3}H]cholesteryl linoleate.

The pathway by which LDL is metabolized in cultured human fibroblasts is shown in Fig. 1. Under conditions of cholesterol deprivation, these cells increase the synthesis of the LDL receptor, which becomes localized to the surface of the cell (*5*). The initial event in cellular LDL metabolism involves the binding of LDL to this receptor. This binding exhibits saturability, high affinity, and specificity. The LDL receptor binds only those human plasma lipoproteins that contain apoprotein B, that is, LDL and very low density lipoprotein (VLDL) (*6, 7*). The binding of LDL to its receptor probably involves an ionic interaction between the protein component of LDL and the surface receptor, which itself appears to be a protein or glycoprotein (*5, 8, 9*). This follows from the fact that LDL binding to the receptor can be prevented and LDL can be dissociated from the receptor by exposure of the complex to heparin (*9*), a sulfated glycosaminoglycan that is known to form soluble complexes with LDL through ionic interactions with its protein component (*10*).

In order to achieve its physiological effect, the LDL that is bound to the receptor enters the cell in a process that resembles absorptive endocytosis. When LDL is bound to the LDL receptor at 4°C under conditions in which endocytosis is minimal, the material remains on the cell surface, as evidenced by its continued susceptibility to release with heparin (*9*). However, when the cells are warmed to 37°C, endocytosis occurs and renders the internalized LDL no longer releasable by heparin (*9*).

The internalized LDL is incorporated into endocytotic vesicles (endosomes) that fuse with lysosomes. Within the lysosome the protein component of the LDL is rapidly degraded to free amino acids, which are then released into the culture medium (*8, 11*). The cholesteryl ester component of LDL is hydrolyzed by a lysosomal acid lipase (*12*), and the resultant unesterified cholesterol is transferred to the cellular compartment where it is found largely associated with cell membranes (*13*). The accumulation of unesterified cholesterol within the cell regulates the activities of two microsomal enzymes: (i) it suppresses 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA reductase), causing a reduction of cholesterol synthesis (*14, 15*), and (ii) it activates an acyl-CoA:cholesteryl acyltransferase, facilitating its own reesterification (*16, 17*). The endogenously reesterified cholesterol is preferentially attached to the monounsaturated fatty acids oleate and palmitoleate, in contrast to the cholesteryl esters of plasma LDL which are rich in the polyunsaturated fatty acid linoleate (*18*). The overall effect of the LDL receptor-mediated process is to transfer free and esterified cholesterol from LDL into the cell (*13*) and to produce a switch in the fatty acid composition of cholesteryl esters from a polyunsaturated to a more saturated form (*18*).

Feedback Regulation of the LDL Receptor

If the LDL receptor functions to transfer cholesterol from plasma LDL to cells, then it might be expected that the LDL binding activity would be suppressed when the cells have accumulated adequate amounts of cholesterol. Recent studies indicate that the activity of the LDL receptor is, in fact, regulated by just such a feedback mechanism (*5*). Incubation of fibroblast monolayers with LDL progressively reduces the ability of the cells to bind ^{125}I-labeled LDL at the high affinity receptor site. The reduction in binding appears to be due to a decrease in the number of LDL receptors. From measurements of the rate of decline in ^{125}I-labeled LDL binding activity after administration of cycloheximide, it has been calculated that the LDL receptor in fibroblasts has a half-life of about 25 hours. It is likely that LDL reduces ^{125}I-labeled LDL binding activity by suppressing the synthesis of receptor molecules (*5*).

The coordinated manner in which cul-

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tured human fibroblasts regulate their cholesterol content is illustrated in Fig. 2. When cells are deprived of cholesterol (no LDL present), the synthesis of the LDL receptor and HMG CoA reductase is greatly enhanced while cholesterol esterifying activity is suppressed. Under these metabolic conditions, cholesterol for membrane formation is derived both from de novo synthesis and from a net hydrolysis of cholesteryl esters stored within the cell. When LDL is added to the culture medium (initial response to LDL), the lipoprotein is bound at the receptor site, internalized, and degraded to yield unesterified cholesterol. The liberated sterol, in turn, suppresses de novo cholesterol synthesis and stimulates the esterifying system so that excess cholesterol can be stored as cholesteryl esters. When sufficient cellular cholesterol has accumulated (steady state with LDL present), synthesis of the LDL receptor becomes suppressed, blocking further cholesterol transport into the cell and protecting against an abnormal intracellular accumulation of sterol. In the usual steady state when cultured cells are growing in medium containing whole serum, cholesterol synthesis is suppressed and the cells preferentially take up and utilize LDL-derived cholesterol, adjusting their LDL receptor activity so as to maintain a constant and normal intracellular level of the sterol (*5, 13*). It is only under extreme conditions, such as when cells are deprived of LDL or when they lack the LDL receptor, that they increase their ability to synthesize cholesterol so as to provide themselves with sufficient sterol to support cell growth.

The discovery of the LDL receptor and an understanding of its actions appears to explain the critical earlier observations of Bailey and co-workers and of Rothblat and Kritchevsky and their co-workers, who first demonstrated that mammalian cells cultured in the presence of animal serum synthesize little cholesterol but are able to utilize exogenous cholesterol contained within the culture medium (*19*).

Mutations in the Pathway of LDL Metabolism

Analysis of the events mediated by the LDL receptor has been facilitated by study of fibroblasts derived from patients with several different clinical disorders of cholesterol metabolism, each resulting from a mutation in a single gene. The mutation which has proved to have the greatest explanatory potential is the one found in patients with the receptor-negative form of homozygous familial hypercholesterolemia (*20*). These mutant cells, which lack functional LDL receptors, fail to bind and take up the lipoprotein with high affinity (*5, 6, 8, 9*) and therefore fail to hydrolyze either its protein or cholesteryl ester components (*8, 11, 12*). As a consequence, LDL does not suppress HMG CoA reductase activity (cholesterol synthesis) (*15, 21, 22*) nor does it activate the acyl-CoA:cholesteryl acyltransferase (cholesterol ester formation) in these cells (*16, 17, 22*).

Figure 3 shows the correlation between the known metabolic effects of LDL in fibroblasts as revealed by studies in which monolayers were incubated with the appropriately labeled LDL at varying concentrations. In normal fibroblasts, all of the secondary metabolic processes—that is, cellular LDL uptake (Fig. 3B), apoprotein B hydrolysis (Fib. 3C), cholesteryl ester hydrolysis (Fig. 3D), suppression of HMG CoA reductase (Fig. 3E), and activation of cholesteryl ester formation (Fig. 3F)—reach a maximum when the cell surface LDL receptor is saturated; that is, at an LDL concentration of about 50 μg/ml (Fig. 3A). The dependence of all these metabolic events on the initial binding of LDL is further illustrated by the finding that all are severely diminished in the homozygous familial hypercholesterolemia fibroblasts, which lack the LDL receptor (Fig. 3).

Confirmation that defective cell surface LDL binding is the primary defect in the homozygous familial hypercholesterolemia cells has come from the following ad-

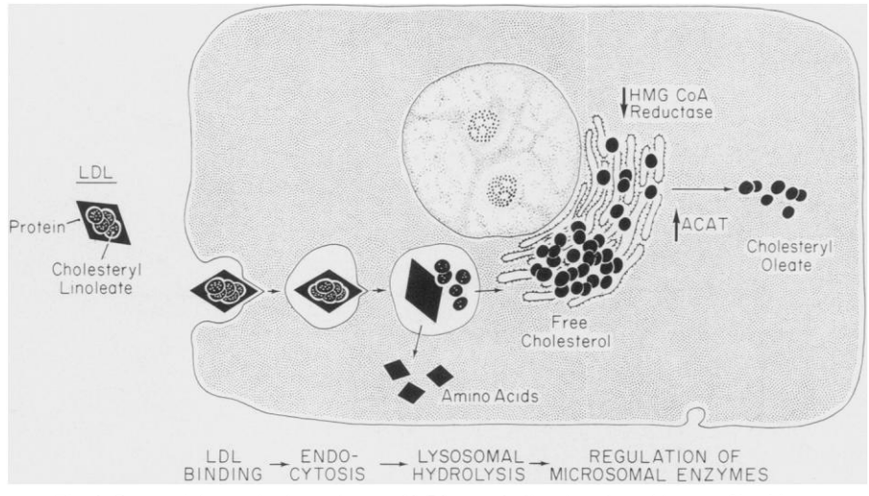

Fig. 1. Sequential steps in the pathway of LDL metabolism in cultured human fibroblasts.

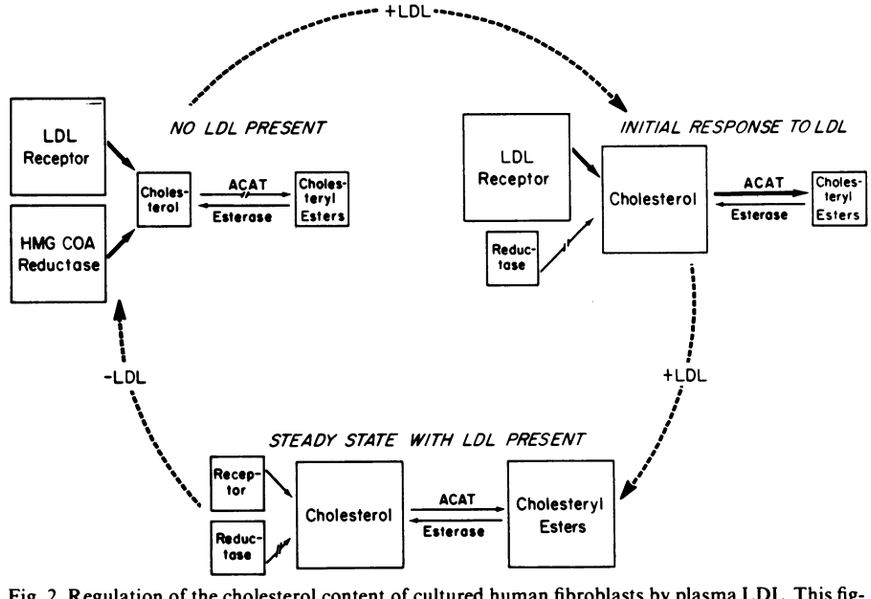

Fig. 2. Regulation of the cholesterol content of cultured human fibroblasts by plasma LDL. This figure indicates the cyclic changes in cholesterol metabolism that occur when LDL is removed from the culture medium (-LDL) and is subsequently returned to the medium (+LDL). The relative cellular level of each constituent is indicated by the size of the square. Abbreviation: ACAT, fatty acyl-CoA: cholesteryl acyltransferase.

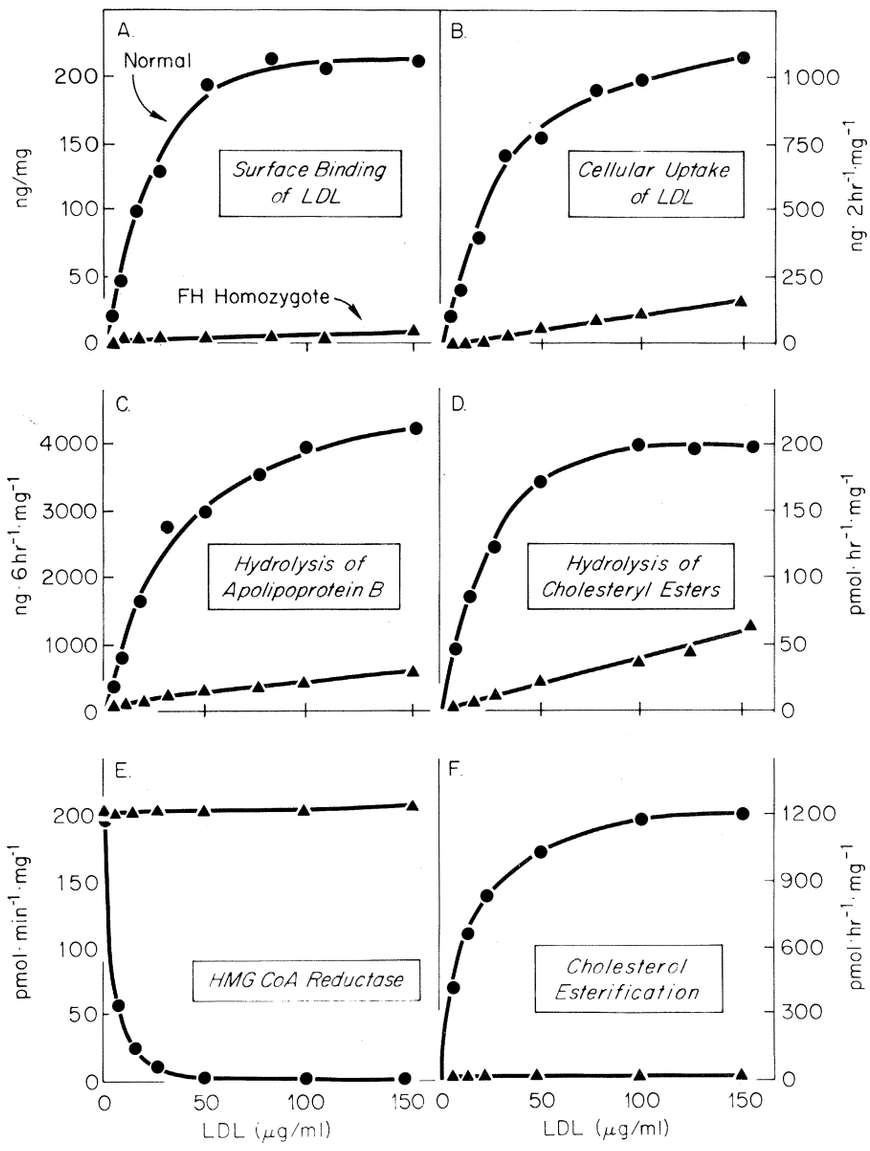

Fig. 3. Manifestations of LDL-receptor interactions in normal (•) and homozygous familial hypercholesterolemia (▲) fibroblasts incubated with varying concentrations of LDL. Cell strains derived from explants of skin were maintained in monolayer culture (*15*), and were set up for experiments (day 0) in 60-mm petri dishes at a concentration of 1×10^5 cells per dish in medium containing fetal calf serum, as described in (*12*). On day 6, which was 48 hours before the experiment, the medium was replaced by medium containing 10 percent human lipoprotein-deficient serum (5 mg of protein per milliliter) (*15*). On day 8, the medium was replaced with 2 ml of fresh medium containing (A to C) ^{125}I-labeled LDL (441 count/min per nanogram of protein), (D) [^{3}H]cholesteryl linoleate–labeled LDL (32,830 count/min per nanomole of cholesteryl linoleate, or (E or F) unlabeled LDL. After incubation with LDL at 37°C for either 2 hours (A and B) or 6 hours (C to F), the indicated measurements were made. (A and B) Surface binding and cellular uptake of ^{125}I-labeled LDL. Each cell monolayer was washed six times at 4°C with an albumin-containing buffer (*8*), and a solution containing sodium heparin (10 mg/ml) was added to each dish. The dishes were then incubated at 4°C for 1 hour; the heparin-containing medium was then removed, and the amount of ^{125}I-labeled LDL bound to the cell surface and hence accessible for heparin release was determined (*9*). The cells were dissolved in 0.1*N* NaOH and the amount of ^{125}I-labeled LDL that had entered the cell and was hence resistant to heparin release was determined (*9*). (C) Proteolytic hydrolysis of ^{125}I-labeled LDL. The medium was assayed for ^{125}I-labeled trichloroacetic acid–soluble degradative protein products that had been formed (*8*). (D) Hydrolysis of LDL-cholesteryl esters: The cellular content of unesterified [^{3}H]cholesterol formed from the hydrolysis of [^{3}H]cholesteryl linoleate–labeled LDL was measured as described (*12*). (E) Suppression of HMG CoA reductase activity. Cells were harvested, detergent-solubilized extracts were prepared, and enzyme activity was determined (*15*). (F) Stimulation of cholesteryl [^{14}C]oleate formation. One hour before the end of the incubation (that is, 5 hours after the addition of LDL), each cell monolayer was labeled at 37°C with 0.1 m*M* [1-^{14}C]oleate (21,000 count/min per nanomole) bound to albumin, and the cellular content of cholesteryl [^{14}C]oleate was determined (*16*). In all experiments, each value represents the mean of duplicate incubations and measurements.

ditional observations. (i) When these mutant cells are broken and cell-free extracts are prepared, their lysosomal enzymes are indistinguishable from normal in their ability to hydrolyze both the protein and cholesteryl ester components of LDL, indicating that the defective hydrolysis in the intact cells is due solely to a failure of the lipoprotein to reach the lysosome (*12*). (ii) When purified cholesterol or one of its oxygenated derivatives (7-ketocholesterol or 25-hydroxycholesterol) is added to these mutant cells in a nonlipoprotein form that allows entry into the cell in the absence of the LDL receptor, HMG CoA reductase activity becomes suppressed (*1*) and the acyl-CoA:cholesteryl acyltransferase becomes activated normally (*17*). (iii) As is expected from genetic considerations, fibroblasts from heterozygotes with familial hypercholesterolemia show about one-half the normal number of LDL receptors (*23*). Thus, a mutation in a single gene involving the LDL receptor can result in a pleiotropic series of abnormalities, each of which is related simply to the inability of the cell to bind LDL at the receptor site.

Just as the familial hypercholesterolemia mutation has been useful in elucidating the role of the cell surface receptor in the LDL pathway, two other genetic diseases—cholesteryl ester storage disease and the Wolman syndrome—have proved helpful in defining the role of the lysosome in this process. In each of the autosomal recessive disorders, the primary defect involves a deficiency in lysosomal acid lipase activity (*24*). Thus, cell-free extracts from cholesteryl ester storage disease fibroblasts show only 5 percent of the normal ability to hydrolyze LDL-bound cholesteryl esters (*18*), and cell-free extracts from Wolman syndrome fibroblasts show an even more pronounced deficiency in the same enzymatic activity (*25*). When LDL is added to intact fibroblasts cultured from patients with either mutation, the lipoprotein binds to the LDL receptor and is internalized in a normal manner (*18*). Moreover, the protein component of LDL is hydrolyzed as rapidly as in normal cells. However, because of the lysosomal acid lipase deficiency, the hydrolysis of the LDL-cholesteryl esters is markedly slowed (*18*), and as a result intact LDL-derived cholesteryl esters accumulate in the cell proximal to the metabolic block, that is, within the lysosome (*25*). The reduced rate of hydrolysis of the LDL-cholesteryl esters produces a marked delay in the transfer of unesterified cholesterol from the lysosome into the cellular compartment, and this is correlated with both a delayed suppression of HMG CoA reductase and a reduced activation of the cholesterol esterifying system (*18*).

Additional evidence for the essential role of the lysosome in LDL action has come from the use of the drug chloroquine, an agent that inhibits a variety of lysosomal hydrolytic processes in intact cells (*26*). The inhibition of lysosomal hydrolysis of LDL-cholesteryl esters by chloroquine prevents both the LDL-mediated suppression of HMG CoA reductase and activation of cholesteryl ester formation (*11, 12, 18*). When the cholesteryl ester storage disease fibroblasts, the Wolman syndrome fibroblasts, and the chloroquine-treated normal fibroblasts are provided with either cholesterol or one of its oxygenated analogs (7-ketocholesterol or 25-hydroxycholesterol) in a nonlipoprotein form, all of these cells suppress HMG CoA reductase and activate cholesteryl ester formation normally (*11, 18*). This provides further evidence that their defective response to LDL is merely a consequence of their reduced ability to generate unesterified cholesterol from LDL within the lysosome.

Receptor-Independent Cholesterol Uptake

In addition to the LDL receptor-mediated pathway shown in Fig. 1, cultured human fibroblasts are able to take up cholesterol from lipoproteins by two other processes. The first involves a simple exchange of unesterified cholesterol between the lipoprotein and the plasma membrane (*27*). In contrast to the receptor-mediated mechanism, this exchange process produces equal rates of cellular uptake and loss of cholesterol so that no net change in the cellular cholesterol content occurs (*13, 27*). Moreover, since this process shows no specificity for either the lipoprotein species or the type of plasma membrane involved, its physiologic role is unclear.

The second form of cholesterol uptake occurs as a passive consequence of the ingestion of droplets of culture medium by the cells during bulk phase pinocytosis. By this process a soluble molecule, such as a lipoprotein, is taken up at a rate that is strictly proportional to its concentration in the medium (*28*); at high lipoprotein concentrations high rates of lipoprotein uptake can be achieved (*8, 11, 29*). When LDL is taken up by such bulk phase pinocytosis, it reaches the lysosome where its protein and cholesteryl ester components are hydrolyzed in a manner similar to that which occurs during the receptor-mediated uptake process (*8, 12*). However, in striking contrast to the latter process, the unesterified cholesterol released from LDL during the bulk phase pinocytotic process does not appear to be capable of expanding the nonlysosomal cellular compartment. Rather, this sterol is excreted into the culture medium (*12*), and thus it neither suppresses HMG CoA reductase nor activates cholesteryl ester formation (*8, 16*). Moreover, lipoproteins taken up by bulk phase pinocytosis do not produce a net accumulation of either free or esterified cholesterol within the cell (*13*).

In fibroblasts from patients with the homozygous form of familial hypercholesterolemia, uptake of LDL cholesterol by the passive exchange process and by the bulk phase pinocytotic process proceed normally (*8, 13*). However, since neither of these processes can produce an increase in the cholesterol content of the cell (*13*), HMG CoA reductase is not suppressed and cholesterol esterification is not activated.

Postulated Role of the LDL Receptor in vivo

If the LDL receptor functions in nonhepatic cells in the body as it does in cell culture, then its existence may help to explain an important concept in cholesterol metabolism that has emerged from the work of Siperstein, Dietschy, Wilson, and others: namely, that more than 90 percent of the total amount of cholesterol produced in the body is synthesized in the liver and intestine even though virtually all nonhepatic tissues both require cholesterol and possess the enzymatic capacity to synthesize it (*30*). We propose that cholesterol synthesis is suppressed in nonhepatic tissues in vivo because these tissues preferentially take up and utilize LDL cholesterol through the LDL receptor mechanism. According to this formulation, the high rate of cholesterol synthesis in liver and intestine is due, at least in part, to the demand for sterol to be incorporated into lipoproteins so that it can be transported and distributed to body tissues.

In view of this division of function between the liver and nonhepatic tissues, it is unlikely that hepatic cholesterol synthesis would be regulated directly by an LDL receptor with the characteristics of the receptor in peripheral cells. Indeed, it is known that the hepatic cholesterol content and hence hepatic cholesterol synthesis are controlled predominantly by dietary cholesterol that reaches the liver in the form of chylomicron remnants rather than by endogenous cholesterol contained in LDL (*30*). The uptake of chylomicron remnants by the liver is presumed not to require the LDL receptor since patients with the homozygous form of familial hypercholesterolemia, who lack LDL receptors and hence have an impaired ability to clear plasma LDL, do not show an impaired ability to clear chylomicron remnants.

Thus far, the LDL receptor has been identified functionally in several cell types examined in culture, including cultured human aortic smooth muscle cells (*31*), cultured human lymphocytes (*32*), HeLa cells (*33*), mouse L cells (*33*), as well as human fibroblasts. That this receptor functions in vivo as it does in cultured cells is suggested by recent observations. (i) Fogelman, Popjak, and co-workers have shown that cholesterol synthesis and HMG CoA reductase activity are low in human leukocytes freshly isolated from blood (*34*). However, when these cells are incubated for 12 hours in the absence of plasma lipoproteins, both activities increase and this increase is prevented by the inclusion of lipoproteins in the incubation medium (*34*). (ii) In patients with abetalipoproteinemia, in whom there is a genetic absence of circulating plasma LDL (*35*), fresh skin slices show rates of cholesterol synthesis that are about fivefold higher than in normal subjects (*36*). (iii) Steinberg and co-workers have shown that catabolism of plasma LDL in swine is not apparently reduced by functional hepatectomy, suggesting that nonhepatic tissues may constitute a major site for LDL degradation in vivo (*37*). Considered together these observations support the proposal that the suppressed cholesterol synthesis observed normally in vivo in nonhepatic tissues is due not to an intrinsic limitation in enzyme capacity, but rather to an active mechanism of regulation that involves the LDL receptor.

The role of the LDL receptor as envisioned above is also in keeping with the emerging concept that plasma lipoproteins not only function to solubilize lipids but also contain within their protein structure the specific information that dictates the body sites to which each lipid is to be delivered. These new insights into cholesterol and lipoprotein physiology will hopefully stimulate further advances into the nature of the pathologic abnormalities that underlie human atherosclerosis.

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Isolable Oxysulfuranes in Organic Chemistry

Many new types of isolable tetracoordinate organosulfur(IV) species are now available.

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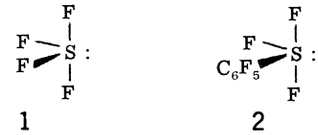
Compounds of sulfur(IV) in which four ligands are attached to sulfur have in common with the rare gas compounds, such as XeF_2, an electronic structure involving a formal expansion of the valence shell of the central atom from eight to ten electrons. We call such compounds sulfuranes (*1*); in this article we discuss those sulfuranes (*2*), only recently available for study, that have oxygen-centered ligands attached to sulfur. The incorporation of oxygen ligands makes possible a wide range of new structural types that illustrate structure-reactivity relationships in a particularly illuminating way.

The possible importance of oxysulfuranes and related species in organic chemistry was highlighted before their isolation by numerous lines of evidence (*3*) pointing to their involvement as high energy intermediates in reactions of sulfoxides, sulfonium ions, and sulfides.

Halosulfuranes

Until recently the evidence for most types of sulfuranes postulated as intermediates was only indirect. The outstanding exception to this generalization is the class of sulfuranes with halogen ligands to sulfur. The halosulfuranes have been known as isolable compounds for many years. The preparation of the very unstable SCl_4 by Michaelis and Schifferdecker (*4*) in 1873 was followed in 1911 by the discovery (*5*) of the thermally stable but reactive sulfurane SF_4 which was not, however, fully characterized until 1929 (*6*, *7*). This was the forerunner of a whole family of stable fluorosulfuranes, derivatives of SF_4 in which one or two fluorines have been replaced by aryl or perfluoroalkyl groups (*8*–*10*).

The geometry of SF_4 (**1**), as determined by microwave spectroscopy (*11*) and electron diffraction (*12*), can be described as distorted trigonal bipyramidal with two fluorines and the lone pair of electrons occupying equatorial positions, with the other two fluorines in apical positions.



1 2

A substituent less electronegative than fluorine, such as the pentafluorophenyl group of **2**, shows a strong preference for an equatorial position in competition with fluorine (*10*) in accord with the general rules describing orders of apicophilicity in pentacoordinate compounds, which were enunciated several years ago (*13*).

The chlorosulfuranes are, in general, less stable thermally than their fluoro analogs. The treatment of sulfur(II) species such as diaryl sulfides with chlorine gives an adduct dichlorosulfurane, **3**, in a reversible reaction which maintains an appreciable vapor pressure of Cl_2 over the dichlorosulfurane at room temperature (*14*).

$$Ar_2S + Cl_2 \rightleftharpoons Ar_2SCl_2$$

3

At temperatures below –30°C, however, such compounds as SCl_4 (*4*), CH_3SCl_3 (*15*), and $(p\text{-}ClC_6H_4)_2SCl_2$ (*16*) are stable. An x-ray crystallographic structure for the latter showed it to be covalent in the crystal with

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