

Thematic review series: *The Pathogenesis of Atherosclerosis*

An interpretive history of the cholesterol controversy, part V: The discovery of the statins and the end of the controversy¹

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Abstract The first four reviews in this series (Steinberg, D. 2004. *J. Lipid Res.* 45: 1583–1593; Steinberg, D. 2005. *J. Lipid Res.* 46: 179–190; Steinberg, D. 2005. *J. Lipid Res.* 46: 2037–2051; Steinberg, D. 2006. *J. Lipid Res.* 47: 1–14) traced the gradual accumulation of evidence, evidence of several different kinds, supporting the lipid hypothesis. They tracked the history from Anitschkow's 1913 classic work on the cholesterol-fed rabbit model to the breakthrough 1984 Coronary Primary Prevention Trial, the first large, randomized, double-blind primary intervention trial showing that decreasing blood cholesterol (using cholestyramine) significantly reduces coronary heart disease events. At that point, for the first time, decreasing blood cholesterol levels became an official national public health goal. Still, only a small fraction of patients at high risk were getting appropriate cholesterol-lowering treatment, and a number of important clinical questions remained unanswered. This final review in the series traces the early studies that led to the discovery of the statins and briefly reviews the now familiar large-scale clinical trials demonstrating their safety and their remarkable effectiveness in reducing coronary heart disease morbidity and mortality.—Steinberg, D. An interpretive history of the cholesterol controversy, part V: The discovery of the statins and the end of the controversy. *J. Lipid Res.* 2006. 47: 1339–1351.

Supplementary key words inhibition of cholesterol biosynthesis • triparanol • compactin • lovastatin

The first four reviews in this series (1–4) chronicled the several lines of evidence that supported the lipid hypothesis, including the dietary intervention trials in the 1960s. By 1970, many leaders in atherosclerosis research were firmly convinced that cholesterol lowering would work. However, dietary intervention had only limited effectiveness and compliance was not easy to effect. Consequently, interest in pharmacological approaches began as early as the 1950s and ultimately led to the discovery of the statins and the end of the controversy.

DECREASING PLASMA CHOLESTEROL LEVELS BY INHIBITING ENDOGENOUS CHOLESTEROL BIOSYNTHESIS

Years before the causal relationship between blood cholesterol level and coronary heart disease risk was widely accepted, there was already considerable interest in the possibility of using drugs to decrease cholesterol levels, especially in patients with markedly increased levels and strikingly high risk. A comprehensive 1962 review of activity in this field listed quite a few agents, some already in clinical use, but most still on the drawing boards (5). Practitioners had precious few choices available, and the efficacy of what was available was limited. Because treatment once started would presumably be for a lifetime, the notion of using drugs at all seemed somewhat quixotic, but the notion of using drugs that would inhibit cholesterol biosynthesis seemed even more quixotic. Skeptics pointed out, quite rightly, that the cholesterol molecule is crucially important as an essential component of all cell membranes and also as an obligatory precursor for the synthesis of steroid hormones and bile acids. Would not these vital functions be compromised, leading to unacceptable toxic side effects? Well, possibly so, especially if inhibition were to be complete or nearly so. But might it not be possible to titrate dosage to inhibit lipoprotein production without compromising those functions for which cholesterol was essential?

That was the gist of the proposal put forward in the early 1950s by Jean Cottet and his collaborators in France (6, 7) and by Steinberg, Fredrickson, and Avigan in the United States (8, 9). However, neither group came up with an effective compound. The drug introduced by Cottet and coworkers (α -phenylbutyric acid) did slow the rate of incorporation of radioactive acetate into cholesterol, but it did not actually decrease de novo production of cholesterol molecules. That is because the compound inhibited the activation of free acetate to acetyl CoA (10) but none

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of the later steps in cholesterol synthesis. Actually, the activation of acetate is not essential for endogenous cholesterol biosynthesis, a point often lost sight of. The degradation of all major foodstuffs (fatty acids, carbohydrates, and some amino acids) generates acetyl CoA, not free acetate. The acetyl CoA deriving from any of these pathways can then serve directly as the starting point for cholesterol synthesis (i.e., without being first degraded to free acetate). Therefore, inhibition of the conversion of acetate to acetyl CoA would not necessarily compromise net cholesterol production, and indeed it did not. The reported cholesterol-lowering effects of Cottet's compound in animals and humans could not be confirmed (11–13).

Steinberg, Fredrickson, and colleagues, following up on observations made by Tomkins, Sheppard, and Chaikoff at Berkeley (14), confirmed that a close chemical relative of cholesterol, δ -4-cholestenone, could inhibit cholesterol synthesis and went on to show that it reduced blood cholesterol levels (9, 15). However, feeding the compound caused the accumulation of cholestanol (16), which was itself proatherogenic, and toxic side effects of the compound precluded clinical use (15). These early efforts failed to solve the problem, but they did at least spark interest in the possibility that cholesterol synthesis might be a legitimate pharmacologic target.

THE MER/29 (TRIPARANOL) SCANDAL: A SETBACK IN THE QUEST FOR DRUGS INHIBITING CHOLESTEROL BIOSYNTHESIS

In the mid 1950s, while randomly screening its chemical library for compounds that might decrease blood cholesterol levels, the Wm. S. Merrell Co. in Cincinnati came across a compound that looked very promising. In rats and dogs it appeared to decrease cholesterol levels by as much as 20–25%. It was assigned the in-house identifier "MER/29" and the generic name triparanol, under which it was later marketed. The company demonstrated that the drug was an inhibitor of cholesterol biosynthesis (17). Later studies at the National Institutes of Health (NIH) established the exact site of action of the drug: it inhibited the very last step on the synthetic pathway, the conversion of desmosterol to cholesterol (18), by reduction of the side chain double bond. Knowing the site of inhibition led to a number of findings that challenged the value of triparanol. For example, it was shown that large amounts of desmosterol accumulated in the plasma of treated animals and patients, accounting for up to 30% of total plasma sterols (19). A key point of confusion was that the color yield of desmosterol in the then standard Liebermann-Burchard reaction was approximately half that of cholesterol. Consequently, the total sterol level in the blood (the sum of cholesterol and desmosterol) was being underestimated and the apparent decrease in cholesterol level was being overestimated. Furthermore, it was shown that desmosterol entered atherosclerotic lesions just as effectively as cholesterol, not surprising given that it is structurally different from cholesterol by just one double bond

(20). Therefore, even though triparanol did have a modest effect in decreasing blood cholesterol levels, that effect was significantly less than it appeared to be; moreover, the accumulating precursor would probably substitute nicely for cholesterol in atherogenesis.

Worst of all, in addition to being relatively ineffective, the drug had serious toxic side effects. It was quickly found that it caused lens cataracts and hair loss in rats and dogs. Rats on high doses actually became blind. Investigators working on the mechanism of action of the drug were well aware of these toxic effects and called them to the attention of the drug company. A group at Merck, Sharp and Dohme formally notified Merrell of these toxic effects several months before the drug was approved by the Food and Drug Administration (FDA) and invited a team from Merrell to visit their laboratories and see for themselves. The invitation was accepted, and three Merrell people visited in January 1961. During the discussions, the Merrell representatives denied having ever encountered cataracts in their own studies but indicated that they would "try to confirm the Merck experiments." It later emerged that Merrell toxicologists had in fact already observed eye damage and even blindness in some of their rats and dogs but failed to include that information in the material they submitted to the FDA. This omission would turn out to be a major factor in the 1963 federal grand jury criminal indictment brought against the company and some of its employees. The company pleaded *nolo contendere*, which protected them against the use of the grand jury findings in subsequent civil suits. Several hundred such suits were filed, and these were settled by Merrell at a cost of ~\$50,000,000. Hard data are not available, but it would not be surprising if the company netted that much during the year they kept the drug on the market, even in the face of the increasing evidence that patients were developing serious eye problems and that the drug was not really decreasing the concentration of blood sterols very much. See R.A. Fine's excellent history of this scandal (21) for more information.

IMPACT OF THE TRIPARANOL DEBACLE ON THE WAR AGAINST CHOLESTEROL

The triparanol fiasco caused many companies to bring to an abrupt halt their hunt for drugs that might block cholesterol synthesis, even though the mechanisms underlying the lens damage and hair loss were not known. Conceivably, the toxic effects might have been attributable to the cholesterol decrease per se and, if so, other inhibitors would then be expected to cause similar side effects. Alternatively, the toxic effects might be unique to triparanol (which turned out to be the case), so that other inhibitors might be free of such effects. However, even if a pharmaceutical house should hit on an inhibitor that did not cause cataracts, there would be the problem of getting FDA clearance. The FDA would predictably now be much tougher and might require, as it should, much more rigorous safety and efficacy data before approving any drugs

in the class. The \$50,000,000 it cost Merrell to settle the civil lawsuits against it surely helped other companies make the decision to “abandon ship.” Fortunately, some drug companies did not completely shut down their research programs on inhibitors of cholesterol biosynthesis. However, most of them decided to steer clear of inhibitors that blocked synthesis at the later stages, hoping that inhibitors working at earlier steps would not share with triparanol the disastrous effects on the eye and on hair growth. Unfortunately, for almost 20 years they came up with nothing usable. Between 1959, when triparanol was introduced, and 1979, when the statin drugs reached the clinic, there were many patent applications for inhibitors of cholesterol synthesis. Not one of them reached the clinic.

THE BIRTH OF THE STATINS: AKIRA ENDO

Much of the following is based on an interview generously granted to me by Dr. Endo, on his own published accounts of the discovery (22, 23), and on the several tributes to Endo in the *Festschrift* published in his honor, especially that by Brown and Goldstein (24). Pharmaceutical companies in the 1970s were panning for antibiotic gold, systematically screening compounds made by fungi and other microbes for their potential as antimicrobial agents. They were inspired of course by Fleming’s discovery of penicillin when one of his Petri dishes sat around too long and got contaminated by a fungus (25). The bacteria originally seeded onto his dish had grown thickly everywhere except for neat, clear circles surrounding the intrusive fungal colonies. Having a prepared mind, Fleming realized that the fungus was making something that killed bacteria in its immediate vicinity, a property that might be put to good use. His discovery was serendipitous, but soon the search for microbial antibiotics became systematized and was being pursued on a large scale.

In 1971, Dr. Akira Endo, working at the Sankyo Co. in Tokyo, speculated that the broths in which fungi were being grown in the hunt for new and better penicillins just might also contain natural inhibitors of cholesterol synthesis. There was at the time no direct evidence to support that speculation, but Endo states that he hoped that some microorganisms might “produce such compounds as a weapon in the fight against other microbes that required sterols or other isoprenoids for growth” (23). Parenthetically, it should be noted that Endo’s interest in cholesterol metabolism dated back at least to 1965, when he applied for a fellowship to work at Harvard with Konrad Bloch. Unfortunately, Bloch had no fellowship openings available at the time and Endo went instead to New York, where he spent 2 years as a Fellow at the Albert Einstein School of Medicine working in the laboratory of Dr. Bernard L. Horecker. On returning to Tokyo, Endo and his associate at Sankyo, Dr. Masao Kuroda, began to test fungal broths for their ability to inhibit cholesterol synthesis from labeled acetate in a cell-free system. The assay was straightforward, fast, and cheap. Endo and his col-

leagues began testing in 1971. Week after week, month after month, they patiently applied their assay to these broths, but the results were uniformly and depressingly negative. Two years and >6,000 tests later, they finally came up with a real winner. The culture broth from *Penicillium citrinium* contained a remarkably potent inhibitor of cholesterol synthesis (26, 27), which they isolated and designated ML-236B. They showed that it inhibited the incorporation of acetate but not that of mevalonate into cholesterol. They pointed out that the ML-236B molecule included a domain homologous to hydroxymethylglutamate and thus the presumptive site binding it to the reductase. ML-236B, for historical reasons discussed below, was referred to in the early years as “compactin,” and the name stuck. We shall continue to refer to it that way in this review.

So now Sankyo had a specific inhibitor working at the HMG-CoA reductase step. The next question was whether it would work in vivo and whether it would be tolerated at effective dosages.

Endo’s first tests were done in rats using just single doses, probably because the amounts of compound available were limited. It seemed at first to work, but when given in repeated doses over a longer period of time there was no consistent effect on blood cholesterol levels (28). It looked as if 2 years of work and >6,000 tests had led nowhere. Fortunately, Endo and associates did not give up at this point, as they might have. They went on to try their compound in dogs, and there the results were quite different; now they saw a very significant and consistent decrease of blood cholesterol levels (29). They also showed that the drug worked in rabbits, hens, and monkeys (30). In retrospect, the reason for the initial “failure” in rats is clear. The drug does actually inhibit cholesterol synthesis in vivo in the rat, just as effectively as it does in other species, even though there is a compensatory increase in the amount of reductase enzyme. However, rats have extremely low LDL levels. Most of their plasma cholesterol is in the HDL fraction. Consequently, even a significant percentage reduction of LDL might not show up as much of a reduction in total cholesterol level, which is what was measured in these early studies.

Endo’s results did not draw a lot of attention initially. Partly, this apathetic reception may have reflected the reaction to the triparanol fiasco reviewed above. There was no great enthusiasm in the pharmaceutical industry for another inhibitor of cholesterol biosynthesis in the 1970s. In 1977, Endo presented a paper in Philadelphia at a symposium on Drugs Affecting Lipid Metabolism, a triennial meeting to which all of the major pharmaceutical companies sent representatives. Surprisingly, his presentation was poorly attended. However, the exciting possibilities of compactin were not lost on Michael S. Brown and Joseph L. Goldstein at the University of Texas Southwestern Medical School (24). Within a month of the publication of Endo’s first report on compactin, they had written to Endo to ask for a sample to use in their ongoing studies of the regulation of cholesterol biosynthesis. Endo sent the samples, and they invited him to visit them in Dallas after the

Philadelphia meeting. They compared notes on their experiments done independently in Tokyo and in Dallas, found them to be concordant, and published the results jointly in a 1978 paper in the *Journal of Biological Chemistry* (31). This was an important paper because it described for the first time the huge increase in the amounts of the reductase enzyme induced in cells by statin treatment. Because the statins are competitive inhibitors, the inhibition, which is powerful at the drug concentrations reached within the intact cells, is largely lost when the tissue is homogenized and the cytoplasm greatly diluted for measurement of enzyme activity. These studies were done using human fibroblasts, but the same phenomenon was later reported in hepatocytes. A few years later, the Goldstein/Brown laboratory showed that this huge overproduction of reductase protein, representing an attempt by the cell to overcome the statin inhibition, is accompanied by a huge buildup of endoplasmic reticulum, the organelle in which the reductase resides (32). As a result, the cells look "abnormal," but of course they are not cancer cells. As discussed below, in retrospect, this may be what led the pathologists at Sankyo at a later date to conclude, incorrectly, that high doses of compactin were possibly carcinogenic.

THE EARLY CLINICAL TRIALS OF COMPACTIN

In 1980, Yamamoto, Sudo, and Endo (33) reported that compactin given by mouth at a dose of 50 mg/day decreased cholesterol levels in patients with hypercholesterolemia by an average of 27% (33). In some patients, the decrease was as much as 30 to 35%. A second clinical study in seven patients with heterozygous familial hypercholesterolemia, which is much more difficult to treat, was later published in the prestigious *New England Journal of Medicine* by Mabuchi et al. (34). It showed a highly significant decrease in total cholesterol levels from 390 to 303 mg/dl. There was no doubt now that, barring the possibility of some unsuspected toxicity showing up in larger and longer clinical trials, this drug and others like it were going to be wonder drugs. Akira Endo had inaugurated the statin era (Fig. 1).

AN INSTRUCTIVE FOOTNOTE TO THE DISCOVERY OF THE STATINS

By a most remarkable coincidence, a British group at Beecham Laboratories in the United Kingdom, while searching for antibiotics, independently isolated precisely the same compound as Endo's ML-236B from a different but related mold and at almost the same time (35). They named it compactin. However, the Beecham workers were narrowly focused on antibiotics, and in that arena compactin was not particularly effective, so it was dropped from the Beecham program. They did not appreciate until later, after Endo had published on its potency as an HMG-CoA reductase inhibitor, what a treasure they had had in



Fig. 1. Akira Endo, the discoverer of the first statin drug in 1976 (27).

their hands. Their initial report did not mention the close homology between the lactone ring in compactin and that of 3-hydroxymethylglutarate. Only in 1980, after Endo had already published several papers on the potency of compactin as an inhibitor, did the Beecham group explore its effects on cholesterol biosynthesis in vitro and in rats in vivo. They readily confirmed Endo's results: it was a potent inhibitor (36). However, like Endo, they observed no change in blood cholesterol levels in rats despite a very respectable 70% inhibition of the rate of in vivo cholesterol synthesis. They concluded, quite incorrectly, that if the drug did not work in rats it probably would not work in other species, even though they noted in their discussion that Endo's group had already reported significant decreases of blood cholesterol levels in dogs and monkeys. What they seemed to be unaware of was that most of the blood cholesterol in rats is in the HDL fraction, so that even a significant decrease of LDL might go undetected. They suggested that the cholesterol decrease that Endo had observed in dogs and monkeys might be attributable not to an inhibition of cholesterol synthesis but to some independent, unrelated effect of the drug. They concluded that trying to decrease blood cholesterol by inhibiting cholesterol synthesis was "futile" and dropped the project. Had they gone on and tested it in other species, as Endo had, Beecham might have entered the statin race early on.

MERCK ENTERS THE RACE: ALFRED W. ALBERTS AND P. ROY VAGELOS

Much of the following is based on interviews and personal communications from A. W. Alberts and P. Roy Vagelos and on Vagelos's memoir, *Medicine, Science and Merck* (37). Needless to say, the dramatic clinical findings with compactin, even though limited to a small number of subjects, made quite a stir. Every pharmaceutical company of size soon began screening their microbial cultures not

just for antibiotics but also for inhibitors of cholesterol biosynthesis. Merck, Sharp and Dohme was first out of the gate. Soon after Endo's papers appeared, P. Roy Vagelos, President of Merck Research Laboratories, signed a confidentiality agreement with Sankyo and obtained samples of compactin. Merck researchers quickly confirmed Endo's findings and were astonished at the potency of the drug. Under the direction of Alfred W. Alberts, a longtime Vagelos collaborator who had come with him from Washington University, Merck set out to find its own statin (Fig. 2). Albert's group started screening in October 1978 and was lucky enough to hit pay dirt with sample 18, just 2 weeks into its program (38).

Quite a contrast to Endo's experience, who screened ~6,000 broths before making a hit!

Alberts's lovastatin had a structure differing by only one methyl group from that of compactin, and it had very similar biological properties. Preliminary clinical studies were begun in 1980, and the early results looked very promising indeed. But the whole Merck program was suddenly shut down in the fall of 1980. The story behind that is an intriguing one, but we need to preface it by going back to Japan and the early work of Endo.

In 1979, Endo was offered an Associate Professorship at Tokyo Noko University and left Sankyo. He continued his pursuit of reductase inhibitors, and in August of the same year he reported the isolation from cultures of a different fungus (*Monascus ruber*) of another highly effective inhib-

itor of cholesterol synthesis, which he named monacolin K. Its chemical structure was very similar to that of compactin, differing only by the addition of a single carbon atom on one of the rings. He applied for a patent in Japan in February 1979.

Meanwhile, Merck was plowing ahead with its own screening program and, as mentioned above, very quickly hit its first promising inhibitor, a compound secreted by a fungus (*Aspergillus terreus*) quite distinct from the one Endo had used. Merck named its compound mevinolin (later changed to lovastatin) and applied for the U.S. patent in June 1979. The remarkable fact is that the structures of Endo's monacolin K and Alberts's lovastatin turned out to be absolutely identical: precisely the same compound produced by two different microbes and discovered independently in two different laboratories almost simultaneously. Endo and his university originally held the patent on monacolin K/lovastatin in Japan but later sold it to Sankyo. Merck held the patent on lovastatin in the United States but did not have worldwide rights. Sankyo was now already quite far along with its clinical studies on compactin, had published several papers on its use in humans, and was probably going to market it any day. Merck was putting every effort into its lovastatin program and had already carried out a few clinical studies. The groundwork was now laid for a knock-down, drag-out race to see who would be the first to successfully bring a statin to market. But then something strange happened.

HOW WE ALMOST LOST THE STATINS

In the fall of 1980, Merck held its usual annual 4 day research "retreat" at which each working group presented its most recent results and its plans for the coming year. That year it was held at the Seaview Resort at Absecon, New Jersey. P. Roy Vagelos, President for Research, had been driven down that morning for the meeting, and he recalls very vividly the dramatic events of that afternoon (Fig. 3). Merck was in excellent financial condition (net income of ~\$400 million) thanks to an innovative drug discovery plan that Vagelos had initiated. That plan had brought several "blockbuster" drugs to the market over the preceding few years. Still, Vagelos knew that maintaining Merck's leadership role required that there be a continuing input of new products into the "pipeline." As Vagelos puts it, "[that's] why we were all watching *Mevacor* [lovastatin] so closely and that's why we were all so upbeat about our research program. We thought *Mevacor* had the potential to become a billion-dollar-a-year product" (37). The day's discussions went well and spirits were high.

Then, toward the end of the day, right in the middle of a wrap-up session, Vagelos was called out to take an urgent phone call from Japan. The call was from the head of Merck's Japanese research office, H. Boyd Woodruff. Sankyo had suddenly terminated all of its clinical studies with compactin. It had given no reasons for this startling move and was unwilling to answer any questions. Woodruff said, however, that rumors were circulating that the com-



Fig. 2. Alfred W. Alberts, who headed the Merck group that isolated mevinolin (lovastatin), the first statin to reach the market and the one to be used in the first critical large-scale clinical trials (36).

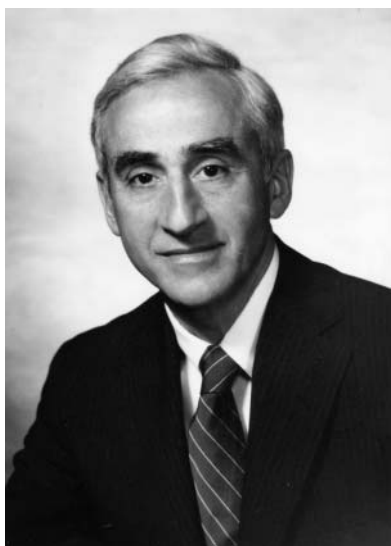


Fig. 3. P. Roy Vagelos, President for Research at Merck at the time mevinolin was discovered and later Chief Executive Officer of the entire corporation. When rumors surfaced that Sankyo's compound might be carcinogenic, he acted responsibly (i.e., he temporarily halted all clinical trials). Careful, long-term toxicity studies at Merck never revealed any carcinogenic potential, and mevinolin got the green light from the Food and Drug Administration in 1987 and led the way into the statin era (35).

pany had discovered intestinal lymphomas in dogs treated with very large doses of compactin over a long period of time. Woodruff had tried to verify the rumors, but the company would not comment. No one seemed to know what was going on. But one thing seemed certain: Sankyo would never have aborted a multimillion dollar program unless it had encountered something really ominous.

What had been a warm and comfortable, even self-congratulatory, company retreat suddenly became something of a wake. Lovastatin differed in structure from compactin by only one carbon atom. If compactin was carcinogenic, it was likely that lovastatin would be also. On the other hand, the carcinogenicity that had allegedly been encountered might be related not to the cholesterol-lowering effect per se but to an unrelated effect of the compactin molecule. Conceivably, the one extra carbon on lovastatin might abolish any carcinogenic potential. However, that was a long shot. Merck was already carrying out studies on the effects of lovastatin in dogs and had not encountered any intestinal cancers, but its studies were of fairly short duration. Longer exposures might confirm the Japanese findings. Merck had already invested millions of dollars on this project. Halting it would mean losing months or years in the race to get its statin drug on the market. Alberts, who had discovered lovastatin, was devoting his energies full time to this project. Jonathan Tobert was well along with safety and efficacy testing in the clinic. Vagelos knew that this might be a real blockbuster drug and that his teams would be devastated if the project was junked. What to do?

Vagelos did the right thing. He would not take any chance of exposing even one patient to a potential car-

cinogen, no matter what it might cost Merck and even if it meant losing the race to be the first to bring a statin to market. He immediately called a halt to all clinical studies and asked investigators to return outstanding samples; he notified the FDA; and he decided to make an all-out effort to get to the bottom of the cancer rumors. At this point, only a small number of patients had received lovastatin and only at low dosages, but still Merck advised its physicians to check carefully for any signs of cancer. None was found, either at that time or later, even after many years of testing in many thousands of patients all over the world. But in the fall of 1980 at Absecon, New Jersey, none of this was known and the mood was somber.

Merck had only rumors to go on, and those rumors were unconfirmed and lacking in detail. How common were these tumors in dogs? At what dosage did they occur? How did that dosage compare with the dosage needed to treat human hypercholesterolemia? Alberts and some of the other Merck investigators wanted to continue at least the animal toxicity studies. A second group favored dropping the whole project and instead making every effort to find a different statin that would be totally "clean" with respect to carcinogenicity.

Vagelos tried every way he could to get more information about the findings that prompted Sankyo to drop its clinical trials, including letters and phone calls to the company's executives asking them to share the results of their safety assessment tests. Sankyo was unwilling to comment. Vagelos did get second-hand confirmation through an American pharmaceutical company that was working with the Japanese that the rumors might be true. So he and Barry Cohen, who was in charge of Merck's international businesses, went to Japan and visited Sankyo themselves. Vagelos offered a business deal: "If you help us solve this problem, we'll share *Mevacor* [lovastatin] with you in Japan and you can share your second-generation product with us when you're ready." The head of Sankyo smiled and said he would like to cooperate but that there were "others" who objected. Vagelos returned empty-handed, puzzled, and angry.

THE IMPLICATIONS OF DROPPING LOVASTATIN

Vagelos was now getting input from investigators who dealt directly with patients with heterozygous familial hypercholesterolemia, patients who could have fatal heart attacks as early as age 30. None of the drugs then available was very effective in these patients. The clinical studies in Japan, although limited in scope, had already shown that these patients would respond to compactin, and for them the drug could be life-saving. Even if there were risks, even life-threatening risks, those had to be balanced against the potential life-saving benefits of treatment. Dropping the program might deny some patients a chance to prolong their lives. Moreover, there was still no hard evidence that compactin would be toxic in humans, only rumors about toxicity in dogs given very high doses. There was no way to be certain about the extent of risk, but the potential ben-

efit could be great in view of the known natural history of coronary heart disease in such families.

This line of argument was urged on Vagelos by a number of clinicians who had used lovastatin in early safety and efficacy trials, including Roger Illingworth of Portland and Scott M. Grundy, David Bilheimer, Joseph L. Goldstein, and Michael S. Brown of Dallas. Two members of Vagelos' Scientific Advisory Board, Jean Wilson from Dallas and Daniel Steinberg from La Jolla, made the same case. In his memoir, Vagelos remembers that "we needed advice from the type of authorities in their field whom the FDA would consult." After these meetings, Merck presented all of its data to the FDA and got a green light for additional clinical trials in high-risk patients. Merck was on its way to putting the first statin drug into the hands of clinicians (the outstanding team at Merck that saw this project to completion included Drs. Georg Alber-Schonberg, Carl Hoffman, James MacDonald, Richard Monaghan, and Arthur Patchett and Ms. Julie Chen).

WAS COMPACTIN INDEED CARCINOGENIC?

The chronic toxicity studies of compactin at Sankyo were done using astronomically high doses: up to 200 mg/kg/day. Yamamoto, Sudo, and Endo (33) had already shown that the dosage needed to decrease cholesterol levels even in severely hypercholesterolemic patients was <1 mg/kg/day. In other words, the dogs were getting ~200 times the dosage that would be used in patients. Still, the toxicologists at Sankyo felt obliged to counsel against continuing the use of even the small dosages being used in patients. Like most Japanese pharmaceutical houses, Sankyo was strongly tilted toward conservatism in the 1960s, partly because of several serious instances of post-marketing toxicity, including the tragic experience with thalidomide. This conservatism tended to be shared generally by the medical profession in Japan at the time. One prominent Japanese clinician warned that "powerful drugs, like a sharp knife, should be considered dangerous." Another warned students not to prescribe drugs at full dosage, thereby running the risk of toxicity, but whenever possible to use half the normal dosage. Another factor may have been the somewhat parochial approach of the pharmaceutical companies in Japan at that time, an unwillingness to openly exchange information with and seek advice from those outside the company "family" (Akira Yamamoto, personal communication). In any case, Sankyo dropped compactin and continued to hunt for other fungal inhibitors.

In retrospect, we can now say with absolute confidence that neither lovastatin nor any of the other statin drugs on the market is carcinogenic, either in experimental animals or in humans. Clinical trials in which tens of thousands of subjects have received either a placebo or a statin have shown no change at all in cancer incidence. In the early 1980s, however, the level of anxiety at both Sankyo and Merck was high, and we came close to losing these wonder drugs.

THE MIRACULOUS POWER OF THE STATINS TO PREVENT HEART ATTACKS AND SAVE LIVES

It is difficult to overstate the impact the statins had on the management of atherosclerosis, particularly coronary heart disease and stroke.

First, because the statins decreased blood cholesterol so much more than any of the existing diet or drug treatments, it suddenly became much easier to demonstrate the decrease in coronary heart disease events and to do so in a statistically significant, unarguable way. For example, in the groundbreaking 1984 NIH Coronary Primary Prevention Trial, using the drug cholestyramine (39, 40), total blood cholesterol in the treated group decreased by only ~10% and LDL cholesterol by ~20%. This was enough to reduce the heart attack rate, but only by ~20%. The result barely reached statistical significance. By contrast, in one of the first large-scale statin trials, total cholesterol was reduced by 25%, LDL cholesterol by 35%, and coronary heart disease deaths by 42%. This reduction was highly significant ($P < 0.00001$). This trial, the so-called 4S study (for Scandinavian Simvastatin Survival Study) in Scandinavia (41), was done using simvastatin, the second Merck statin, which was discovered while the company was assessing the safety of lovastatin. The 4S study showed, for the first time in any cholesterol-lowering trial, a significant decrease in all-cause mortality. A new era in the treatment of hypercholesterolemia and coronary heart disease had arrived.

A recent meta-analysis of 14 statin trials with an astonishing total of 90,056 individuals randomized (using lovastatin, simvastatin, pravastatin, fluvastatin, or atorvastatin) showed that the decrease in coronary events was best predicted by the absolute decrease in LDL levels. The incidence of major vascular events was reduced by ~20% for each 1 mmol/l (40 mg/dl) decrease in LDL cholesterol (42). Thus, an individual starting with an LDL of 280 mg/dl whose level decreased to 200 mg/dl on therapy (a 29% decrease) would be predicted to have a 40% decrease in risk over a 5 year period.

Second, the large-scale statin studies laid to rest the lingering concerns that decreasing blood cholesterol levels might be intrinsically dangerous. This concern arose originally because in the European clofibrate trials there were indeed more deaths in the drug-treated group than in the controls, although the difference was marginal (43). In retrospect, this difference was probably attributable not to the decreased cholesterol level per se but to a toxic effect intrinsic to the clofibrate molecule and unrelated to its cholesterol-lowering activity. The second-generation fibric acids (e.g., gemfibrozil and fenofibrate) have not shared the toxicity of clofibrate (44, 45). Concerns that decreasing blood cholesterol levels might be intrinsically dangerous were misplaced a priori 1) because levels of intracellular cholesterol are jealously guarded by the LDL receptor homeostatic mechanism (46), and 2) because most animal species have LDL levels well below those reached during even the most aggressive treatment of hypercholesterolemia (47). Obviously, these animals' cells

do just fine. Nevertheless, this had been a concern and deterred some physicians from treating hypercholesterolemia vigorously. The large-scale statin trials showed that even decreasing LDL levels to <100 mg/dl was not only safe but actually decreased overall mortality significantly (41, 42, 48–52).

Third, there had been concern that although treating hypercholesterolemia might reduce coronary heart disease risk, it might at the same time lead somehow to increases in mortality from other causes, not necessarily because of the decreased cholesterol levels per se but possibly from metabolic dysfunctions arising from other properties of the cholesterol-lowering agents. Indeed, in the Coronary Primary Prevention Trial, there had been a statistically significant decrease in coronary heart disease mortality and yet no decrease in all-cause mortality. Of course, as was pointed out at the time, the study was not designed to have the power to show a decrease in all-cause mortality; that would have required a larger number of subjects (39, 40). Nevertheless, much was made of a small, statistically nonsignificant increase in the category of “violent deaths,” which included suicides, homicides, and traumatic deaths (e.g., automobile accidents). (How homicides could be made more likely by the victim’s cholestyramine intake was never made clear.) In any case, as first shown in the 4S trial using simvastatin (41) and borne out in the meta-analysis of $>90,000$ subjects in 14 statin trials (42), statins decreased all-cause mortality.

One cause for concern about the safety of decreased cholesterol came from prospective epidemiologic studies. These showed that individuals with low blood cholesterol levels when initially surveyed (e.g., <160 mg/dl) were more likely to die during the next 5 years than those with average cholesterol levels. In retrospect, this was probably attributable to the fact that a number of potentially life-threatening diseases are characterized by low blood cholesterol levels in the early, preclinical stages. This is true, for example, in many forms of cancer and in cirrhosis of the liver. In other words, the poor prognosis in the group with initially low cholesterol levels might be accounted for by the fact that some fraction of them entered the study already ill. The NIH in 1990 convened a panel of experts to discuss the possibility that decreasing cholesterol levels might be intrinsically dangerous. The panel concluded that the evidence did not justify such a finding, but with the data available at that time neither could it be ruled out absolutely (53). The large-scale statin studies settled the issue. It is now clear that the marginal effects on all-cause mortality seen in the early trials were attributable in part to the small sizes of the populations studied and in part to the modest decreases of cholesterol levels.

Fourth, the large-scale statin studies made it clear for the first time that statin treatment benefits 1) women as well as men, 2) the old as well as the young, 3) those with low initial LDL levels as well as those with high initial levels, and 4) diabetics as well as nondiabetics. None of the earlier studies had been large enough to make these benefits evident.

Women

Women before the menopause have a much lower risk of coronary heart disease than men of the same age. However, after menopause, their risk increases, and over a life span coronary heart disease takes just as great a toll in women as in men. Nevertheless, there had been a tendency for physicians to regard women as “immune” and to undertreat their hypercholesterolemia. The statin studies have clearly shown that women benefit just as much from treatment as do men.

The elderly

Until recently, physicians were somewhat reluctant to treat hypercholesterolemia in elderly patients. “Why bother them with yet another pill when they don’t have much longer to live?” Only with the statin studies completed in the past few years has it become apparent that even patients older than 75 years benefit from treatment, in relative terms, as much as younger people. Because the chances of heart attack are much greater in the elderly, the absolute number of heart attacks prevented by treating 70 year old men is even greater than that prevented by treating men a decade or two younger.

In these days when life expectancy has increased to 75 years in men and 80 years in women, the number of good years of life conferred by treatment is large and the treatment is eminently worthwhile.

Patients with near-normal LDL levels

The question of “how high is high” is a complicated one (54). The 2001 Adult Treatment Panel III guidelines for physicians advised intensive treatment of very high-risk patients (e.g., those with established coronary heart disease or with high risk as a result of diabetes), treatment designed to decrease their LDL to 100 mg/dl (55). Some earlier studies had suggested that there was no significant reduction in risk for patients with an initial LDL level of 125 mg/dl or less (51). However, in the British Heart Protection Study, using simvastatin, subjects with initial LDL levels of <100 mg/dl, the “goal” by then current standards, showed a significant further reduction of LDL levels and a further significant reduction of coronary heart disease risk with statin treatment (48). Some epidemiologic studies, particularly studies in Chinese populations, had previously shown that coronary heart disease risk decreased with cholesterol levels even when the total cholesterol levels were in the 120–160 mg/dl range (i.e., LDL levels of ~ 60 –100) (56). However, the Heart Protection Study was the first to demonstrate directly that decreasing LDL levels even below the 100 mg/dl level, previously considered to be ideal, does indeed confer additional benefit. The percentage reduction in risk was approximately the same as that in subjects with higher initial LDL levels. Very recently, using high doses of statins, it was shown that decreasing LDL to a mean of 79 mg/dl arrested progression (measured by intravascular ultrasound), whereas decreasing it only to a mean of 110 mg/dl still allowed further progression (57).

Overviews of the statin trials (42, 52) show clearly that the lower the plasma LDL on treatment, the lower the incidence of major end points (Fig. 4). Almost certainly, these findings will result soon in a further reduction in the “goal LDL levels” recommended by the National Cholesterol Education Program.

Diabetics

Most diabetic patients die of coronary heart disease, not coma or microvascular complications. For reasons still unclear, atherosclerosis proceeds at a higher rate in these patients, and heart attacks occur about a decade earlier. When the diabetes is under good control, the LDL levels are not necessarily increased but a low HDL is the rule. So it was not certain that decreasing LDL levels with statins would be effective. The 4S study (58) and the British Heart Protection Study (48) provided the answer, and their results were confirmed and extended in subsequent studies. Diabetics, whether with previous coronary heart disease or not, show as much benefit as nondiabetics.

WHAT CAN WE EXPECT IN THE FUTURE WITH THE STATINS?

Atherosclerosis is a disease of multiple etiologies. Proper clinical management should include intervention on all of them: dyslipidemia (high LDL, low HDL), cigarette smoking, hypertension, obesity, diabetes mellitus, lack of exercise. Yet, intervention on just one risk factor, increased LDL, has reduced coronary heart disease risk by 30–40% in the 5 year statin studies, indicating that hypercholesterolemia is a dominant determinant of clinical expression. What about the 60–70% of treated subjects who have a coronary event despite statin therapy? Several points can be made.

1) First, except for some very recent studies, the dose of statins used in clinical trials has been less than the maximum and less than optimal. Nor were adjuvant antilipid therapies included in an effort to obtain the maximal LDL decrease. Even this less than ideal intervention has reduced event rates dramatically. For primary prevention

(Fig. 4), the prediction from extrapolation is that with an on-treatment LDL level of ~57 mg/dl, there might have been no events (52). We recognize that extrapolations like this are not really justified, and we have tongue firmly in cheek. Still, the data suggest that we may not yet have reached the limit of what can be achieved just by decreasing LDL. With simultaneous attention to other causative factors, the impact should be even greater.

2) Second, these studies have for the most part lasted for only 5 years. Percentage reduction in event rate might be significantly greater after 10 or 15 years of treatment.

3) Third, almost all of the trials to date have been done in subjects with an average age of 50–60 years. We know that the arteries of these subjects harbor well-developed lesions even if they have no clinical manifestations of atherosclerosis. What, then, if intervention was started at age 40 or even 30, when the lesions are fewer and smaller? By how much would such early intervention further reduce the event rates? In individuals at unusually high risk, treatment should be started even earlier, even in childhood. A randomized, double-blind study of children ages 8–18 years has demonstrated a significant slowing of intimal thickness in the carotid artery and no adverse effects on growth, hormone levels, sexual maturation, or liver function (59). In short, the impact of the statins may ultimately exceed considerably that demonstrated in the clinical trials to date. However, if we hope to reach our goal of zero tolerance for myocardial infarction, we are probably going to have to start treatment earlier and also combine LDL-lowering with equally vigorous attention to the other treatable risk factors. New modes of intervention under intensive current study include 1) increasing HDL or otherwise favoring reverse cholesterol transport (60), 2) inhibiting cholesterol absorption from the intestine (61, 62), and 3) attacking the inflammatory and immune processes contributing to the arterial lesion (63–65).

WHY DID THE CHOLESTEROL CONTROVERSY LAST SO LONG?

Throughout this series of reviews (1–4) and in an earlier analysis of the controversy (66), we have identified a num-

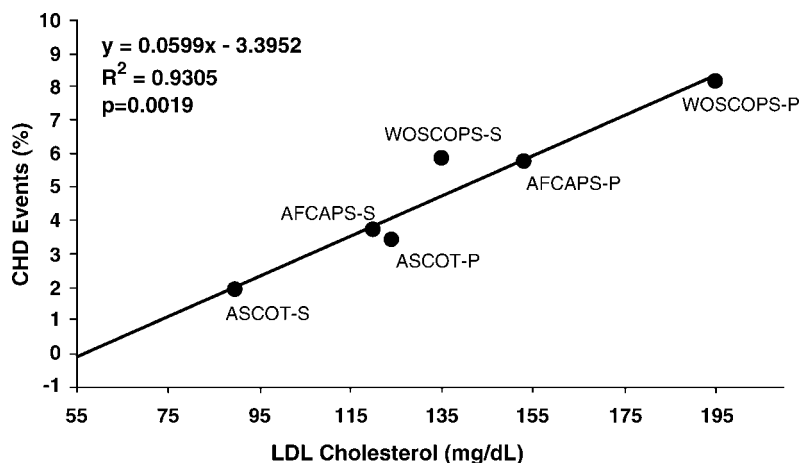


Fig. 4. Pooled data from primary prevention statin trials, plotting the coronary heart disease (CHD) event rates during the trials as a function of LDL cholesterol levels during the trials (P, on placebo; S, on statin). The dramatic decrease in event rates as LDL decreases is evident. Extrapolation of the data might suggest that the event rate would decrease to zero at ~57 mg/dl LDL. Of course, such extrapolations are not warranted, but the fact that national guidelines for LDL-lowering are approaching just such a level is at least suggestive. Reproduced from O’Keefe et al. (52) with permission from the American College of Cardiology Foundation.

ber of reasons why the proposal to treat hypercholesterolemia was so strongly resisted for so long. Those reasons include the following.

1) Dismissal of Anitschkow's rabbit model and other animal models as not relevant to the human disease.

2) The misguided search for a single cause to a complex disease of multiple etiology. The argument was: "If only a subset of cases show hypercholesterolemia, then hypercholesterolemia cannot be a major causative factor." Closely related to this reason is the next.

3) Confusion regarding what constitutes a "normal" blood cholesterol level: unwillingness to accept the notion that a very large fraction of our population actually has an unhealthily high cholesterol level.

4) Undue concentration on the advanced, complex lesions rather than on the initiating factors.

5) Confusion between cholesterol in the diet and cholesterol in the blood. Of course, it is the latter that counts; the diet is relevant, but mainly as one of the determinants of blood cholesterol.

6) The reluctance of practitioners in the 1970s and 1980s to grapple with the (to them) still elusive plasma lipoproteins and their complex metabolism.

7) The limited and relatively unsatisfactory dietary and drug regimens available for controlling hypercholesterolemia before the statin era. ("What's the difference? We can't do much about hypercholesterolemia anyway.")

8) The absence, until fairly recently, of a consensus on the detailed mechanisms linking cholesterol and lipoproteins to the damage in the artery wall.

9) The preoccupation of cardiologists with their new and exciting diagnostic and interventional tools, and impatience with the notion of preventive cardiology.

10) Most important of all, resistance to the need to synthesize evidence of several different kinds (epidemiologic evidence, experimental observations in animals, genetic evidence, clinical observations, and clinical trial data) in evaluating the true strength of the lipid hypothesis. The early clinical trial results, although weaker than might have been desired, were nevertheless impressive when looked at in the context of all of the other lines of evidence.

SOME THOUGHTS ON HYPOTHESIS TESTING

A major message from the history presented in this series of Thematic Reviews is item 10 in the list above: that cumulative evidence and evidence of different kinds should be taken into account in evaluating postulated causal relations and certainly must be taken into account when deciding what to do about them. The lipid hypothesis proposed that hypercholesterolemia was a causative factor in human atherosclerosis. It did not propose that hypercholesterolemia was the only cause but that it was at least a quantitatively significant factor. An implied corollary was that appropriate intervention to correct hypercholesterolemia might reduce the rate of progression of atherosclerosis and its clinical manifestations. To many researchers and clinicians, the only definitive test of the

lipid hypothesis was going to be the clinical trial: the gold standard single-variable, randomized, double-blind, placebo-controlled intervention trial. And that indeed is the crucial test of the corollary. However, results of any clinical trial need to be evaluated in the light of prior information bearing on the likelihood of the hypothesis. For example, let us say that a clinical trial of cholesterol-lowering yields a 20% decrease in event rate with a P value of 0.07. To a skeptic (or to one unfamiliar with the many other lines of evidence supporting the lipid hypothesis), such a result ($P > 0.05$) might be the death knell of the hypothesis. On the other hand, to one familiar with the extensive ancillary evidence supporting the hypothesis, the same result would probably be regarded as importantly supportive. It would at the very least lead to additional studies and might even lead to recommendations that treatment of high-risk patients be considered. [Space does not allow a discussion of the widespread misunderstanding of the true meaning of the P value and its limitations; the interested reader will find clear expositions in the papers by Peto et al. (67) and Goodman (68).]

The notion of weighing all of the findings bearing on a hypothesis rather than looking at the clinical trial results in isolation seems self-evident. It was actually formally put forward >200 years ago by Bayes and has been extended and formalized in a number of ways over the years (69, 70). Unfortunately, we still do not have a consensus on the Bayesian approach, nor the instruments to formally quantify the weights of different lines of evidence for inclusion in a probability algorithm. Much as we would like to avoid subjective weightings in the evaluation of biomedical hypotheses, to reach sound judgments regarding health policy we simply have to use all of the evidence available. The cholesterol controversy could have been resolved much earlier if all of us had looked at all of the evidence.

SUMMARY

Some of the key milestones leading to the ultimate acceptance of the lipid hypothesis are summarized in **Table 1**.

The advent of the statins, reviewed above, made it possible to settle the cholesterol controversy once and for all. The message from the pioneering dietary intervention trials, reviewed in Part II of this series (2), and from the Coronary Primary Prevention Trial with cholestyramine (39, 40), reviewed in Part IV (4), was confirmed and importantly extended by the statin trials, as reported here. No one any longer doubts the wisdom of decreasing blood cholesterol. Extrapolating from the exciting results of the 5 year statin studies already reported, we can safely predict that when treatment is started earlier and continued for a longer time, heart attack rates will decrease even more strikingly. Hopefully, early and intensive medical attention to hypercholesterolemia and the other risk factors will eventually reduce sharply the need for interventional cardiologic procedures.

As a result of the statin studies, the ideal LDL cholesterol level for subjects at high risk has decreased to ~ 70 mg/dl

TABLE 1. Milestones on the road to acceptance of the lipid hypothesis

1913: Anitschkow, the cholesterol-fed rabbit model
1939: Müller, familial hypercholesterolemia, xanthomatosis, and angina pectoris linked to hypercholesterolemia
1949: Gofman, the lipoproteins of human plasma and their correlation with the risk of coronary heart disease
1952–4: Kinsell and Ahrens, blood cholesterol in normal subjects is increased by saturated fats in the diet
1955: Keys' Seven Country Study: incidence of coronary heart disease directly correlated to hypercholesterolemia and to dietary fat intake in an international epidemiologic survey
1961: Framingham Study: within a typical American community, coronary heart disease risk is highest in groups with highest blood cholesterol levels
1961: American Heart Association first endorses the "prudent" low-fat diet
1964: Nobel Prize to Konrad Bloch for elucidating the pathway for cholesterol biosynthesis, paving the way for the statins a decade later
1966–9: Leren, Miettinen, Dayton, and others: reducing blood cholesterol levels by reducing saturated fat intake reduces coronary heart disease risk; however, data are not considered persuasive
1974: Goldstein and Brown, the LDL receptor and regulation of cholesterol and lipoprotein metabolism
1976: Endo, discovery of the first effective statin drug (statins not marketed until 1987)
1980: Merck, discovery of mevinolin (lovastatin), later to become the first statin to reach the market
1984: Lipid Research Clinics' Coronary Primary Prevention Trial, which started in 1971, shows significant reduction in coronary heart disease primary events in hypercholesterolemic men treated with cholestyramine
1984: National Institutes of Health (NIH) Consensus Conference on Lowering Blood Cholesterol to Prevent Heart Disease: NIH, for the first time, declares decreasing blood cholesterol to be a national public health goal
1984: NIH coordinates a national program to teach physicians and patients how to diagnose and deal with dyslipidemia, the National Cholesterol Education Program
1985: Nobel Prize to Brown and Goldstein for groundbreaking work on the regulation of cholesterol and LDL metabolism
1994: The statin era: Scandinavian Simvastatin Survival Study, the first truly large-scale, randomized, double-blind trial to show that aggressive treatment (with simvastatin) not only reduces coronary heart disease mortality but also decreases all-cause mortality
1995–present: A stunning series of studies demonstrating the power and the safety of statin intervention

(71). Physicians are being urged to be more aggressive, using higher doses and using combination drug therapy. Except for rhabdomyolysis, an extremely rare side effect, and an increase of transaminase occasionally requiring discontinuation, the statins have proved to be remarkably safe, safer even than aspirin (72). Some have proposed, only half in jest, that we put them "in the drinking water" as we do fluoride. In the United Kingdom, simvastatin (10 mg tablets) is already available over the counter; we in the United States are not ready to go quite that far (73). However, it is noteworthy that in this new statin era, such proposals are no longer "unthinkable." ■

First and foremost, the author is indebted to the Editors, Edward A. Dennis and Joseph L. Witztum, for inviting this somewhat unusual series of reviews. Contemporary histories of science may seem superfluous to those living the history but may contain points of view valuable to those who will put it into future contexts. The author thanks the Editors for providing

the space to develop some thoughts on this historic controversy, which will be expanded in *The Cholesterol Wars*. Thanks also to Akira Endo, Albert W. Alberts, P. Roy Vagelos, Joseph L. Goldstein, and Michael S. Brown for their valuable input and for critiquing the final manuscript of this last review in the series. Finally, thanks to so many colleagues with whom the author has enjoyed discussing (and sometimes fuming about) the great cholesterol controversy over the past 50 years.

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