Foundations for blockbuster drugs in federally sponsored research

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ABSTRACT ‘Blockbuster’ drugs, which are widely prescribed and improve the health of millions, often originate in fundamental laboratory research. An important example of such drugs are the cholesterol-lowering drugs called ‘statins’, including Zocor, Pravachol, and Lipitor, which millions of people take in the U.S. every year. This short paper outlines the direct and indirect contributions of federally sponsored research to the development of these important drugs.—Thompson, R. B. Foundations for blockbuster drugs in federally sponsored research. FASEB J. 15, 1671–1676 (2001)

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Fundamental biomedical research benefits the nation by providing the knowledge base for the discovery and development of new medicines. Particularly in the case of ‘blockbuster’ drugs, which are very widely prescribed (annual sales of greater than $1 billion), the impact is great not only for the health of patients, but also economically. Using the ‘statin’ family of HMG CoA reductase inhibitors that act to reduce serum cholesterol (Zocor, Mevacor, Lipitor) as an example, this paper describes various ways that federal research support provides the foundation that has made these medicines possible.

Millions of Americans now worry about their cholesterol, especially how much they consume in their diet and how much is present in their blood. However, it is only relatively recently that the importance of cholesterol to the development of heart disease was identified. Even now the molecular mechanisms of most cardiovascular disease remain to be fully described. In fact, cholesterol had been isolated in the 1800s and was known to be an important constituent of animal cell membranes; by the 1930s, it was established as the precursor to hormones like testosterone as well as the bile salts. Cholesterol is a lipid, a fatty molecule that dissolves in liquids like olive oil but not in water. Cholesterol was suspected to be important in cardiovascular disease almost 90 years ago, when it became known that the arteriosclerotic ‘plaques’, which actually clog the arteries, contained substantial cholesterol (1) and that feeding some animals a high-cholesterol diet accelerated the appearance of cardiovascular disease (2). Despite these early indications, there was no proof that cholesterol was more than a bystander in the process of arteriosclerosis.

The Framingham heart study established that cholesterol levels in the blood were important in heart disease. This was a very large prospective study beginning in 1948 that examined 5209 residents of Framingham, Massachusetts, looking for risk factors for atherosclerosis leading to heart attack and stroke. The study was funded primarily by the National Institutes of Health (NIH). The study consisted of frequent, thorough physical examinations and extensive blood testing of the volunteers over periods extending to decades. A key risk factor identified in the study was a high level of serum cholesterol (3). High levels of other serum lipids (fatty compounds) such as triglycerides generally were not correlated with a higher risk for heart attack. Naturally, this suggested that reducing serum cholesterol might reduce atherosclerosis and thus the risk of death from heart attack, which, in fact, is the case (4).

Large, long-term studies such as the Framingham study are expensive: the Framingham study has cost more than $27 million just since 1983 (5). The cost is perhaps understandable when one considers that in the early 1970s, more than 10,000 people participating in the study were receiving medical exams that were much more frequent and thorough than ordinary medical exams. The cost of those exams and laboratory tests, together with the expense of collecting and analyzing the data, can run into thousands of dollars per patient. Studies like Framingham are also a gamble because there is no guarantee the study will find an answer. It was far from certain that a risk factor for heart disease would be found or that, if found, it would be useful for treatment or prevention. Ultimately, nearly a dozen risk factors were identified during the course of the study: high serum cholesterol is merely the best known and most therapeutically important. The high cost, multi-year duration, and uncertain payoff necessarily make such studies seem unattractive and unrealistic for the private sector. Only the federal government has been willing to provide adequate funding for the duration of

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the Framingham study, as well as hundreds of other research studies and clinical trials aimed at the reduction of cardiovascular disease. The federal investment has paid off handsomely; it identified a therapy that has proved effective for millions of people—saving lives as well as billions of dollars in health care costs.

One potential way of lowering serum cholesterol is by limiting intake in the diet. Most meat and dairy foods provide amounts of cholesterol adequate for dietary needs. The body needs about 1 gram of cholesterol every day to replace that lost primarily through the digestive tract. Controlling dietary intake has limited success in lowering cholesterol not only because of the difficulty some patients have in staying on a diet, but also because the body can biosynthesize all the cholesterol it needs. Vegetarians who do not consume animal tissue obtain essentially no cholesterol from their diet, yet they do not lack for the cholesterol they require because their own bodies can make it.

The biosynthesis of cholesterol was elucidated over a period of more than 20 years beginning in the late 1940s principally by groups led by K. Bloch of Harvard University, F. Lynen of the Max Planck Institute in Munich, J. W. Cornforth of the National Institute for Medical Research in London, and G. Popják of UCLA, with much of the work in the U.S. supported by the National Heart Institute, the progenitor of the current National Heart, Lung, and Blood Institute of the NIH (6). Bloch, Lynen, and Cornforth later won Nobel prizes for their work. Again, this extensive series of experiments represented a large investment in essentially pure science, since it was not known at the time that reducing cholesterol would be therapeutically important. Indeed, even with the more powerful biochemical tools now available, elucidating such a biosynthetic pathway remains a substantial scientific effort.

The ‘biosynthetic pathway’ for cholesterol is a series of chemical reactions in the cell that convert and assemble the precursor molecules into the final product, cholesterol. The assembly is carried out by a series of enzymes, each of which catalyzes one step. For instance, the first step (of more than 20) is the coupling of two precursor molecules, which each contain two carbon atoms to make one containing four carbon atoms; cholesterol has 27 carbon atoms in all. The biosynthesis of cholesterol is complex compared with that of other small molecules and requires a lot of energy. In part because of the energetic cost, the biosynthesis in the cell (most cholesterol biosynthesis in the body occurs in the liver) is under tight control exerted early in the synthetic process, before much energy is consumed. The tight control is analogous to a thermostat: an automatic system that turns off a furnace when the proper temperature is reached, maintaining the temperature at a more or less constant level. The step in cholesterol biosynthesis where control is exerted is an enzyme called hydroxymethyl glutaryl coenzyme A reductase (HMG CoA reductase, for short), which catalyzes the reduction of HMG-CoA (the ‘substrate’) to mevalonic acid (the ‘product’). Figure 1. The step catalyzed by HMG CoA reductase is essentially irreversible in that it is difficult for the body to convert mevalonic acid back to HMG CoA. However, HMG-CoA can be readily recycled to a precursor, which is used in synthesizing other fatty molecules. This step in the biosynthesis is referred to as the ‘committed step’. Thus, the search began for potential drugs that might stop the biosynthesis process, preferably at the HMG CoA reductase step. The therapeutic importance is twofold: 1) the reversibility of the prior steps means that no potentially toxic precursors would build up if the biosynthesis were halted at that stage; 2) the body itself controls biosynthesis at this step, so any potential intervention would mimic the body’s own function and thus be less risky.

An effective way to halt a pathway like cholesterol biosynthesis is to add a chemical blocker, called an inhibitor, that will interfere with an enzyme that catalyzes a step in the pathway and prevent its function. Many drugs, including aspirin, act as enzyme inhibitors. The way to tell whether a particular chemical is an inhibitor is to see how it affects the activity of the enzyme: more precisely, one measures how much product the enzyme makes in the presence and absence of the inhibitor. The potency of an inhibitor is indicated in green and 6-position (X) in blue. Note that other substrates of the reaction have been omitted and the substrate, product, and inhibitor structures are shown in their ring-opened rather than lactone forms, both for the sake of clarity.

Figure 1. HMG CoA reductase reaction and inhibitors. The upper panel indicates the structures of HMG CoA (substrate) and mevalonic acid (product) of the HMG CoA reductase-catalyzed reaction, with the hydroxymethyl glutarate portion of HMG CoA and mevalonate in red. The lower panel indicates the structures of several HMG CoA reductase inhibitors, with their differing substituents at the 2’ position (Y) indicated in green and 6-position (X) in blue. The step in the biosynthesis is referred to as the ‘committed step’. Thus, the search began for potential drugs that might stop the biosynthesis process, preferably at the HMG CoA reductase step. The therapeutic importance is twofold: 1) the reversibility of the prior steps means that no potentially toxic precursors would build up if the biosynthesis were halted at that stage; 2) the body itself controls biosynthesis at this step, so any potential intervention would mimic the body’s own function and thus be less risky.

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CoA reductase (or any enzyme) requires a ready source of the enzyme to test against and a simple assay for measuring how fast the enzyme converts HMG CoA into mevalonic acid in the presence of the inhibitor. Again, the substantial investment NIH and the National Science Foundation (NSF) made in fundamental studies of enzyme purification, assay development, kinetic analysis, and enzyme function beginning as early as the 1950s has provided the basis of technology and understanding that made it feasible to search for such inhibitors. Thus, in 1960 Durr and Rudney devised a straightforward method of isolating the HMG CoA reductase from baker’s yeast as well as a simple assay for the enzyme activity, which made it possible for anyone to test a particular chemical to see whether it was an inhibitor (7). Often, inhibitors are molecules very similar to the natural substrate(s) of an enzyme, but ones the enzyme cannot convert into a product; the penicillins are examples of such inhibitors. Beginning in the early 1970s, a number of inhibitors were found, but few were potent or specific or could be administered to animals and induce a reduction in cholesterol levels (8, 9).

At about this time, Akira Ando and colleagues at SANKYO Pharmaceuticals in Japan began testing the fermentation broth of molds and other microorganisms for HMG CoA reductase inhibitors. Many simple organisms and plants produce chemicals that are toxic or noxious to discourage animals from eating them; examples of this strategy include poisonous mushrooms and hot peppers. A sufficiently potent HMG CoA reductase inhibitor might be quite toxic to noncarnivorous animals that obtain no cholesterol in their diet. Fermentation technology is well developed in Japan and is a potentially rich source of pharmaceuticals. Ando’s group isolated about a teaspoonful of a compound they called ML-236b (see Fig. 1) from more than 800 gallons of fermentation broth of a mold species related to the one that produces penicillin. ML-236b was a potent inhibitor of HMG CoA reductase, able to shut down the enzyme activity when present at microgram per liter levels (10). Moreover, when administered to rats it induced a significant reduction in their serum cholesterol (11). Brown and colleagues in Britain isolated and characterized the same compound at about the same time as an antifungal compound and named it compactin; they did not suspect that it was an HMG CoA reductase inhibitor, but the name stuck (12). The similarity of the portion of the inhibitors highlighted in red to HMG CoA and mevalonic acid can easily be seen (Fig. 1); many enzyme inhibitors are molecules that are structurally similar to the substrate or product of the reaction.

Unlike the earlier compounds, compactin showed real potential as a drug for reducing cholesterol levels due to its potency and lack of overt toxicity in mammals. As a result, several groups began to isolate and synthesize related compounds, including mevinolin, lovastatin, and pravastatin (see below). Several of these compounds ultimately entered wide use as anticholesterol drugs. In the case of a molecule like compactin, the amount isolated from the fermentation broth was enough to permit compactin’s structure to be determined (12) but not to test it as extensively as is necessary. Knowing the structure, however, it is usually feasible to synthesize the molecule in the test tube in amounts large enough to test. Much of the chemical technology developed for synthesizing these compounds in the laboratory and determining their structure has also been developed with the support of NIH and NSF. As a result, a large ‘chemical toolbox’ is available that permits essentially any molecule known to be made synthetically. Similarly, determination of the structure of small molecules like compactin has become straightforward due to technology enhancements in X-ray crystallography and infrared and NMR spectroscopy, developed largely with federal grant support. Although these techniques have important practical applications, their development was aimed primarily at answering research questions. Perhaps most important to the whole enterprise are the skilled biomedical scientists and biochemists who do the work, a large fraction of whom are supported by NIH during their predoctoral and postdoctoral training in this country. Before support from NIH became available, Ph.D.’s in the biomedical sciences were a rarity. Even though it may be argued whether too few or too many biomedical scientists are currently being trained in the U.S., there is no question that the American pharmaceutical and biotechnology industries (which lead the world) could not exist, let alone thrive, without those thousands of trained people.

To be therapeutically useful, a drug must do more than simply inhibit an enzyme or exert some other therapeutic effect. It must be otherwise nontoxic, or at least there should be a large difference between levels in the bloodstream that are therapeutically useful and those that are toxic. Beyond this, there should be a minimum of side effects associated with the drug. Ideally, the drug should be absorbed rapidly enough and eliminated from the body slowly enough to make only one or a few doses a day necessary. All these issues come into play after a potentially useful compound is identified. Many times it is necessary to modify the structure of the drug to achieve the desired pharmacological attributes. Typically, medicinal chemists will make or isolate many compounds similar to the original, in search of a better molecule. In the case of compactin, scientists at Merck isolated a compound that differs only slightly from compactin (Fig. 1), but is more effective (13). This compound was mevinolin, sold as Mevacor. Subsequently, Merck also introduced lovastatin (Zocor).

Zocor is now among the most widely prescribed drug in existence, accounting for sales in 1998 of 4.7 billion dollars a year worldwide, or ~40% of the market (14). Other important ‘statins’ include Lipitor from the Parke-Davis division of Warner-Lambert (now Pfizer), Pravachol from Sankyo/Bristol-Myers Squibb, and Lescol from Novartis. The production, packaging, quality

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assurance, and marketing of these drugs employ thousands of people here and abroad; Merck alone employs 57,000 people. Export of these drugs also improves the U.S. balance of trade.

The HMG-CoA reductase inhibitors shown in Fig. 1 are all related to compactin; as a result, they share a couple of drawbacks. First, compactin and its relatives are natural products, meaning they are isolated from an organism in nature. Although significant improvements can be made to the rate of production from organisms like molds and fungi, the small amounts of compactin isolated by Ando indicate the enormous amount of effort necessary to scale up fermentation to commercially useful quantities. Like many natural products, compactin can exist in several subtly different configurations, called stereoisomers. Often only a single stereoisomer (of the 256 possible for compactin) is made in nature and is active, with the other possible stereoisomers being inactive. As a result, chemical synthesis of molecules like compactin can be extremely difficult and prohibitively expensive for production purposes.

Recognizing these problems, scientists began to consider whether other, simpler molecules might not also be HMG CoA inhibitors. It is apparent from the red highlighting in Fig. 1 that only a modest portion of compactin actually has a structure similar to mevalonic acid, and perhaps the complex two-ring structure of compactin is not really required. As structural information on enzymes such as HMG CoA reductase became available in the 1980s, scientists working with the support of NIH and industry began to use these structures to understand how current inhibitors worked and help design new inhibitors. In particular, scientists developed something called ‘quantitative structure activity relationships’ (mercifully abbreviated as QSARs) to judge the potential efficacy of inhibitors. At about this time, powerful computer graphics programs were developed at the University of California at San Diego and elsewhere that permitted the 3-dimensional structures of enzymes and inhibitors to be displayed and manipulated in real time. These developments were important because it was no longer necessary to synthesize a series of related molecules to find the best inhibitor; one could essentially create models of the proposed inhibitor molecules in the computer and bring them together with the structure of the enzyme to predict whether a particular molecule would inhibit the enzyme. An image of a portion of human HMG CoA reductase is depicted in Fig. 2 (15). These software programs were an outgrowth not only of biomedical research, but also of computer science research funded by several agencies, including NSF and the Department of Defense. This approach was much faster and cheaper than actually finding or making and testing many new inhibitors. In the mid-1980s Sandoz developed fluvastatin and the Parke-Davis division of Warner Lambert developed atorvastatin. Both these compounds are HMG CoA reductase inhibitors that differ much more from compactin than Mevacor (see Fig. 3). The advantage of these compounds (from a production and therefore profitability standpoint) is they are much simpler: each has only four possible stereoisomers and, consequently, is dramatically cheaper to produce.

More recently, the entire process of creating potential medicines (called leads) and testing them for efficacy has become highly automated, which has led to revolutionary increases in efficiency and cost effectiveness. For a century, drug companies had identified potential drug candidates by collecting thousands of field specimens of plants/animals and testing them (or their constituents) for efficacy in treating disease. The

Figure 2. Structure of human HMG CoA reductase fragment. Reproduced with permission from ref 15.

Figure 3. Structures of new HMG CoA reductase inhibitors atorvastatin and fluvastatin, with the ‘mevalonate’ portions of the molecules indicated in red.
National Cancer Institute of the NIH also invested heavily in a search for antitumor drugs in the same way, identifying many of the anticancer drugs currently in use. After they had identified an active compound (as in the case of compactin), chemists synthesized piecemeal a series of similar chemicals whose structures differ slightly from the parent and could be tested for improved efficacy, reduced side effects, improved uptake and persistence, and other desirable properties. This piecemeal synthesis and testing are still very expensive and inefficient, even when guided by known enzyme structures and QSARs.

Beginning in the early 1990s, several chemists with NIH support began to devise means of systematically synthesizing scores or hundreds of analogs essentially simultaneously (16, 17); this ability is termed ‘combinatorial chemistry’. At the same time, other workers began to devise means of testing very large numbers (into the millions) of compounds for efficacy (17). For instance, instead of synthesizing one or a few analogs of compactin and testing them one after the other for their ability to inhibit HMG CoA reductase, a ‘library’ of thousands of compounds could be synthesized and tested within months, essentially automatically. An important advantage of the combinatorial approach is that potential new drugs can be found without knowing much about their target in the cell or what the structure of an inhibitor should be like. This approach has revolutionized the pharmaceutical development business, making it much easier and cheaper to identify potential drug candidates. For instance, during the 60 years from 1934 to 1994, Merck’s scientists synthesized, purified, and screened for therapeutic efficacy ~250,000 different chemicals at huge expense. Using combinatorial synthesis and high throughput screening techniques, Merck scientists in the 4 ensuing years synthesized and tested 4.5 million compounds (14). This approach was developed principally in the U.S. with NIH support, and the U.S. leads the world in the development and implementation of this technology. Indeed, providing the instrumentation, chemical reagents, and software to implement the combinatorial approach is now a multibillion dollar business worldwide (18), one that is dominated by American firms. It is unclear whether the combinatorial approach is currently being used to find HMG CoA reductase inhibitors, but it clearly is now the principal route to new medicines of all kinds, particularly inhibitors of enzymes newly identified during the sequencing of the human genome.

Thus, at the present time we have a group of medicines that reduce serum cholesterol levels by up to 40%, which substantially reduces the risk of heart attack and other consequences of atherosclerosis such as stroke and kidney failure (4). These drugs are generally well tolerated by those taking them and typically need to be taken only once a day. As a result, they are used by millions all over the world, providing substantial benefits in combating disease and enhancing the quality of life. The production, distribution, and marketing of these drugs comprise a multibillion dollar business internationally, employing thousands of persons in this country and contributing favorably to America’s foreign trade balance. It should be evident that it would have been difficult or impossible to develop and produce these drugs in the absence of federally sponsored research, especially without the knowledge base and substantial number of trained scientists it provides. This has made our research enterprise an important national asset, one we should encourage and support for the future.

In the interest of brevity, many important references describing seminal work could not be included; we apologize to the many workers whose work is not cited. The author wishes to express his gratitude to David Brautigan for the original concept for the study, as well as his help and encouragement; Johann Deisenhofer for Fig. 2; Wolfgang Mergner, Kim Collins, Mark Jenkins, and Richard Engel for critical reading of the manuscript; and Howard Garrison and Tamara Zemlo of FASEB for critical reading of the manuscript and many helpful suggestions. The author is grateful for support from the National Institutes of Health, Office of Naval Research, and National Science Foundation.

Glossary

Bile salts: Major constituents of bile made in the liver and stored in the gall bladder, bile salts are added to the food in the digestive tract to suspend lipids so they may be absorbed by the body more easily. Essentially, they are biological detergents.

Cholesterol: A steroid molecule that plays three main roles in the body; it is an essential component of cell membranes; it is the precursor from which bile salts are synthesized in the body; and it is the precursor for steroids in the body, such as the hormones testosterone and progesterone.

Enzyme: A protein molecule that catalyzes the transformation of one molecule (the substrate) into another (the product). An enzyme may be one of a series that act in succession to synthesize a molecule like cholesterol from simple precursors. Enzymes are said to catalyze the conversion of molecules called substrate(s) into product(s). There are more than 30,000 different enzymes in the human body.

Inhibitor: A molecule that inhibits the functioning of an enzyme.

Lipid: A fatty molecule used in the body. Lipids are oily or ‘hydrophobic’ molecules that separate from water. Examples are cholesterol and other steroids, phospholipids, and triglycerides.

Steroid: A class of lipid with a characteristic fused ring structure; examples include cholesterol, testosterone, progesterone, and the bile salts.

Triglycerides: A class of lipid molecules that are the main constituent of ‘fat’ in fatty tissue, vegetable oil, and butter. Triglycerides consist of three fatty acids attached to a glycerol backbone; the structure of the fatty acids determines whether the triglyceride is liquid or solid at room temperature.
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