thematic review

Thematic review series: The Pathogenesis of Atherosclerosis

An interpretive history of the cholesterol controversy: part I[1]

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Abstract **This is the first of a series of reviews of the controversy that swirled around the "lipid hypothesis" of atherosclerosis for so many years. Today, in the era of the statins, there is no longer any doubt about the value of decreasing blood cholesterol levels. In fact, "the lower the better" is the position of many clinicians. However, getting to this point has been a long uphill battle marked by heated debate and sometimes violent disagreement. The history of this controversy is worth telling for its own sake and because remembering it may help us avoid similar mistakes in the future.**—Steinberg, D. **An interpretive history of the cholesterol controversy: part I. *J. Lipid Res.* 2004.** 45: **1583–1593.**

Supplementary key words atherosclerosis • plasma cholesterol • lipoproteins • coronary heart disease • N. N. Anitschkow • J. W. Gofman • analytical ultracentrifuge • atherogenic index • experimental atherosclerosis • rabbit model of atherosclerosis

The history of science is studded with controversies, and this is especially true of medical science. Certainly, some measure of skepticism is appropriate. New hypotheses need to be critically tested and new treatments need to be carefully evaluated for efficacy and safety. Therefore, some degree of conservatism is a virtue when it prevents the adoption of inadequately tested new treatments, such as the use of thalidomide in pregnant women. On the other hand, an overly cautious approach and an exaggerated skepticism can delay the introduction of therapies that might save lives. The hypothesis that high blood cholesterol levels contribute causally to atherosclerosis and coronary heart disease (the "lipid hypothesis") was for too many years a victim of exaggerated skepticism.

Today, when "good cholesterol" and "bad cholesterol" are the stuff of cocktail hour chatter, it may come as a surprise that dyslipidemia was not always accepted as a significant factor in atherosclerosis and coronary heart disease. Yet in 1946, Peters and VanSlyke, in their classic textbook, *Quantitative Clinical Chemistry*, summarized their view of the evidence this way: "although there can be no doubt that deposits of lipids, especially cholesterol, are consistent and characteristic features [of atherosclerotic lesions] there is no indication that hypercholesterolemia plays more than a contributory role in their production" (1), not an unfair assessment for 1946. At that time, most physicians considered atherosclerosis to be an inevitable accompaniment of aging, about which nothing much could be done, and nothing much was being done. Over the next four decades, the case against hypercholesterolemia would became ever stronger, based on pathology, clinical observations, genetic studies, and epidemiology. Clinical intervention trials with diet, although small in scale, suggested as early as the 1960s that treatment to reduce hypercholesterolemia would reduce coronary heart disease risk. Based on these emerging findings, the American Heart Association as early as 1961 had already accepted the causal relationship and recommended that people at high risk be advised to modify their diets to avert heart attacks. In 1964, these dietary recommendations were extended to include the general public. In 1965, the Food and Nutrition Council of the American Medical Association made similar recommendations. In 1969, the Chairman of the Council on Arteriosclerosis of the American Heart Association in his Presidential Address said, "It is now good medical practice to treat—and I use the word advisedly—people who have definite hyperlipoproteinemia" (2). However, very few practitioners paid much attention to cholesterol levels, dietary advice was minimal, and drug treatment for hypercholesterolemia was in its infancy.

The evidence for the lipid hypothesis became stronger every year, but the idea that the cholesterol level could be centrally important was rejected, at times quite angrily, by

Manuscript received 5 April 2004.

Published, JLR Papers in Press, April 21, 2004.
DOI 10.1194/jlr.R400003-JLR200

[1] Based in part on *The Cholesterol Wars*, a book in preparation.

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many cardiologists and nutrition experts. In 1976, an editorial in the *British Heart Journal* concluded that: "The view that raised plasma cholesterol is per se a cause of coronary heart disease is untenable."

Sir John McMichael, then the preeminent British cardiologist, attacked the hypothesis in a 1979 article aggressively titled "Fats and Atheroma: An Inquest" (3). Even E. H. Ahrens, Jr., whose own pioneering clinical research showed conclusively that blood cholesterol could indeed be reduced by appropriate changes in diet, took strong exception to proposals to change the diet of the American public. In 1979, he wrote that such recommendations would be "unwise, impractical, and unlikely to lead to a reduced incidence of arteriosclerotic disease" (4). Michael Oliver, for many years a vocal skeptic regarding the importance of blood cholesterol levels, wrote in 1981: "It is probably of little value to reduce raised serum cholesterol concentrations in patients with overt [coronary heart disease]" (5). He also was on record to the effect that "reduction of raised serum cholesterol is a card of uncertain quality in the primary prevention of [coronary heart disease]" and that "reduction of raised serum cholesterol could lead to adverse biological changes" (6). A letter to one of the British health-news newspapers referred to Oliver as one of the "Abominable No-men," and an editorial comment in the *Journal of the American College of Cardiology* took issue with Oliver's negative views and titled the essay "The Cholesterol Pessimist" (7).

But Oliver's skepticism was shared by many others. I. D. Frantz, Jr., and Richard B. Moore summarized the situation very aptly in 1969: "Few controversies have divided the medical community so sharply for such a long time as has the sterol hypothesis. The separation between the two points of view has become so extreme that, on the one hand, there are respected scientists who believe that the evidence is already so convincing that further clinical testing is unnecessary, financially wasteful and actually unethical; and, aligned against them, are equally respected scientists who believe that the total weight of evidence accumulated over the many years is too slight to justify further work along these lines" (8). Sad to say, this was still the case in 1983, 15 years later, despite an ever-increasing number of epidemiologic studies, experimental animal studies, and additional intervention studies indicating a causal relationship between blood cholesterol and coronary heart disease. So, in 1983, we still needed a "you-can't-argue-with-this" type of study, a blockbuster. That was finally provided by the large trial of cholesterol lowering sponsored by the National Institutes of Health (NIH) and published in 1984: the Coronary Primary Prevention Trial. For the first time, we had a large, randomized, double-blind study showing a statistically significant decrease in hard cardiovascular end points as a result of decreasing cholesterol level with the use of a bile acid binding resin, cholestyramine. With those data in hand, the NIH then convened a Consensus Conference to advise on whether decreasing blood cholesterol should become a national therapeutic goal (9). The expert panel agreed unanimously that the accumulated experimental, epidemiologic, clinical, and interventional trial data proved the case. Many if not most investigators warmly endorsed the conclusions of the panel. A lead article in the *Medical Journal of Australia* by Leon A. Simons titled "The Lipid Hypothesis Is Proven" concluded: "The LRC-CPPT has given a new respectability and credibility to the dietary and pharmacologic management of hypercholesterolemia" (10). *Postgraduate Medicine* put it this way: "Coronary disease prevention: Proof of the anticholesterol pudding" (11). Paul Nestel, a leading expert in lipid metabolism, wrote: "Time to treat cholesterol seriously" (12).

However, the Consensus Conference conclusions were vigorously challenged at the time, and for a number of years afterward, by a small but vocal group of colleagues. For example, George W. Mann, associate professor of biochemistry at Vanderbilt University College of Medicine, had this to say about the directors of the NIH-sponsored trial: "They have held repeated press conferences bragging about this cataclysmic break-through which the study directors claim shows that lowering cholesterol lowers the frequency of coronary disease. They have manipulated the data to reach the wrong conclusions." And later: "The managers at NIH have used Madison Avenue hype to sell this failed trial in the way the media people sell an underarm deodorant" (13). Oliver had this to say: "Those who initiated the idea [of the Consensus Conference] were either naïve or determined to use the forum for special pleading, or both. The panel of jurists . . . was selected to include experts who would, predictably, say that . . . all levels of blood cholesterol in the United States are too high and should be lowered. Of course, this is exactly what was said" (14). E. H. Ahrens, Jr., whose pioneering work had clearly shown the important impact of diet on blood cholesterol levels, wrote in *The Lancet*: "The Diet-Heart Question in 1985—Has It Really Been Settled?" (15). In its September 1989 issue, *The Atlantic* published and featured on its cover a blistering attack by Thomas J. Moore entitled "The Cholesterol Myth" (16). Moore, a science journalist, wrote: "the dissenters have been overwhelmed by the extravaganza put on not just by the heart institute [sic] but by a growing coalition that resembles a medical version of the military-industrial complex. This coalition includes . . . the 'authorities' . . . the heart institute [The National Heart, Lung and Blood Institute] itself . . . and the American Heart Association." Moore then went on to name explicitly five investigators very active in the lipid field at the time who had offered to make themselves available to answer questions about the statins, which had just been introduced by Merck for clinical use (Drs. Antonio Gotto, Scott M. Grundy, John LaRosa, Robert I. Levy, and Daniel Steinberg). There followed a series of short paragraphs about this "Gang of Five" (my term) and the arguably actionable conclusion that "It is likely that one reason these physicians consented to such an arrangement is that their laboratories were heavily involved in research funded by Merck." Finally, borrowing heavy-handedly from Marc Antony, he wrote: "There is no reason to doubt the honesty, sincerity, and expertise of any of these men." Yes, the cholesterol controversy has seen its share of vitriol.

This swirling controversy over the years made it an uphill battle to convince practitioners, even including the cardiologists (perhaps especially the cardiologists), to pay attention to hypercholesterolemia. Only after the statins, potent inhibitors of cholesterol biosynthesis with remarkable effectiveness in decreasing cholesterol levels, became available in the late 1980s and 1990s did active treatment of hypercholesterolemia become universal good medical practice. Even Oliver finally accepted the hypothesis and began to proselytize for aggressive treatment (17).

In this series of reviews, I will retrace the cholesterol controversy. I will contend that the importance of blood cholesterol levels in human atherosclerosis should have and could have been appreciated much earlier. I will point out the opportunities that were missed, the findings that went unappreciated because of preconceived mind sets, and, most importantly, the unwillingness on the part of some to weigh not only the results of individual clinical trials but the totality of the evidence.

Today, we know that we are winning the war against coronary artery disease. It can be prevented. In fact, clinical trials with the statins have shown remarkable decreases in both coronary heart disease mortality and also total mortality. Decreasing LDL by ~25% is enough to lower coronary heart disease mortality by 30–40%, and that is the result of only 5 or 6 years of intervention. It seems reasonable to extrapolate and expect even greater reductions if treatment is started earlier in life and continued not for just 5 years but for decades. The history of this remarkable medical achievement is worth retelling for its own sake. More importantly, it is worth telling because we can hope to learn from the mistakes of the past and avoid making them in the future.

This review will be largely confined to the research that ultimately established dyslipidemia (high LDL and/or low HDL) as a causative factor in atherosclerosis and showed that it was a preventable disorder, not an inevitable accompaniment of the aging process. Of course, many factors in addition to dyslipidemia contribute to the atherogenic process and help determine when it will express itself clinically. I will not deal with those in any detail.

This is not a general review of atherogenesis nor a history of lipid research per se. Rather, it is an inquiry into how, after much controversy, cholesterol and lipoproteins were implicated, indicted, and ultimately found guilty.

NIKOLAI N. ANITSCHKOW AND THE CHOLESTEROL-FED RABBIT

In 1913, a young experimental pathologist named Anitschkow,[2] working at the Military Medical Academy in St. Petersburg, showed that simply feeding rabbits purified cholesterol dissolved in sunflower oil induced vascular lesions closely resembling those of human atherosclerosis, both grossly and microscopically (18, 19) (**Fig. 1**). Controls fed only the sunflower oil showed no lesions. It is fair to say that this paper marked the beginning of the modern era of atherosclerosis research. Over the next few years, Anitschkow and his colleagues established the following points (20):

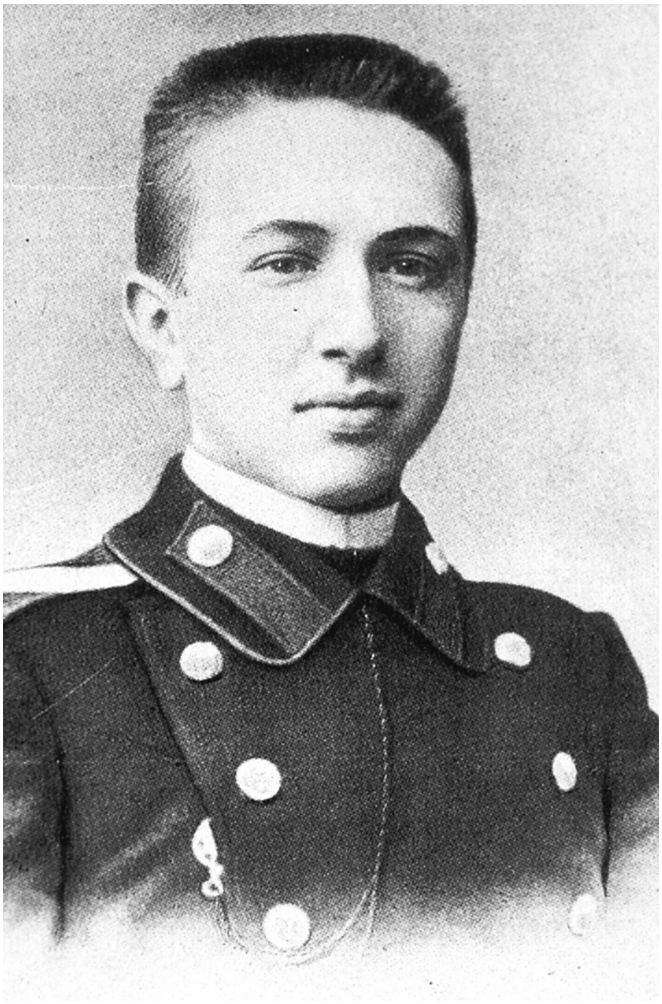

Fig. 1. The young N. N. Anitschkow (circa 1904), at the time a student at the Military Medical Academy in St. Petersburg. Reprinted from *Am. J. Cardiol.* **72:** 1071–1072, 1993 [ref. (24)], copyright (1993), with permission from Excerpta Medica, Inc.

1) That in the earliest lesions, the fatty streaks, most of the lipid was found in cells containing large numbers of lipid-containing vacuoles (foam cells) (**Fig. 2**). These were Sudan positive and contained birefringent droplets (the liquid crystals of cholesterol esters). Note that the endothelium over the lesion appears to be intact.

2) That the very earliest lesions appeared at the root of the aorta and in the aortic arch and then proceeded caudally (**Fig. 3**).

3) That there were characteristic patterns of distribution of early lesions, which were recognized as probably determined by hemodynamic factors (**Fig. 4**).

4) That over long periods of cholesterol feeding there

[2] There is no accepted convention for transliterating Russian names. I chose "Anitschkow" because he himself spelled it that way when he published in English and that is the spelling used by PubMed. Other spellings have included "Anitchkov," "Anitchkow," "Anichkov," "Anichkow," and perhaps others.

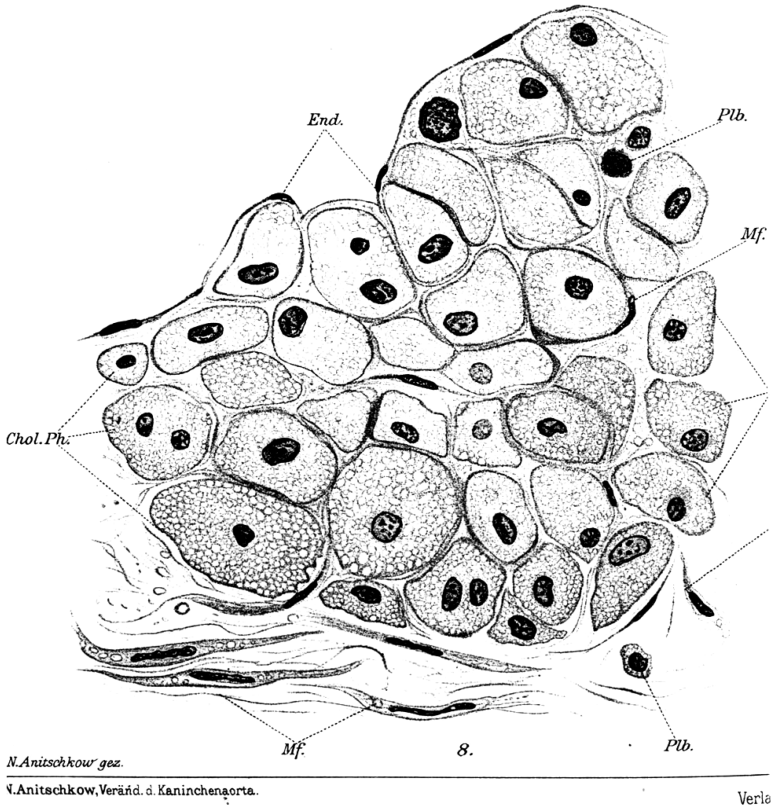

Fig. 2. Anitschkow's drawing of a typical foam cell-rich lesion in a rabbit fed a total of 82.7 g of pure cholesterol in sunflower oil over a period of 139 days. *Chol.Ph.*, large phagocytes filled with anisotropic material; *End.*, endothelial cells; *Plb.*, lymphoid wandering cells; *Mf.*, smooth muscle cells of the aortic wall. [From ref. (19).]

was ultimately deposition of connective tissue (conversion of the fatty streak to the fibrous plaque) and development of a fibrous cap (**Fig. 5**).

5) That early lesions were reversible but that only some of the lipids could be mobilized from advanced lesions, leaving behind the fibrous cap and some cholesterol crystals (**Fig. 6**).

6) That the extent of lesions was proportional to the degree of blood cholesterol increase and the duration of exposure to it.

Furthermore, Anitschkow speculated (correctly):

7) That the "cholesterin" (the name used in Europe for cholesterol at the time) was probably entering the artery wall from the blood;

8) That the cholesterol-loaded cells were probably white blood cells that had infiltrated the artery wall.

Anitschkow was not only a keen-eyed structural pathologist and a careful experimentalist; he thought in terms of function and time-related pathogenesis. If the full significance of his findings had been appreciated at the time, we might have saved more than 30 years in the long struggle to settle the cholesterol controversy and Anitschkow might have won a Nobel Prize. Instead, his findings were largely rejected or at least not followed up. Serious research on the role of cholesterol in human atherosclerosis did not really get under way until the 1940s. Why?

Some laboratories did indeed try to confirm Anitschkow's findings, but instead of using rabbits most of them used the laboratory animals they were familiar with, rats or dogs. Cholesterol feeding in these species failed to induce lesions. So, understandably, these investigators concluded that Anitschkow's results must reflect some peculiarity of the rabbit. After all, it is a strict herbivore that normally has zero cholesterol intake and a very low fat intake. The rabbit model was dismissed as irrelevant to human disease.

What was not appreciated was the fact that rats and dogs, unlike rabbits, are very efficient in converting cholesterol to bile acids. Consequently, even on very high cholesterol intakes the blood cholesterol in these species does not increase appreciably. Steiner and Kendall, 33 years later, would show that inhibiting thyroid function in dogs and then feeding them cholesterol does increase blood cholesterol and then does induce lesions (21). So here was one reason Anitschkow's work was not taken seriously: failure to recognize the two-step nature of what was going on, that is, feeding of cholesterol followed by increases of blood cholesterol levels. Only if the second step kicks in do you get atherosclerosis. Actually, Anitschkow had himself tried to induce lesions in dogs and noted that they did not respond. He speculated that the dog, a carnivore, was adapted to eating fat- and cholesterol-rich foods and could therefore excrete excess cholesterol, a remarkable metabolic insight for the early 20th century (20).

Another reason Anitschkow's findings were not taken seriously is that the blood cholesterol levels in his rabbits were extraordinarily high: 500–1,000 mg/dl or even higher. The argument was that human levels were almost never that high and that extrapolation was unwarranted. This was a legitimate reservation at the time, but soon after his original studies, Anitschkow showed that more modest increases of cholesterol levels in rabbits were sufficient to induce lesions, it just took longer.

Fig. 3. Sudan-stained aorta of a rabbit fed 61 egg yolks over a 70-day period. Anitschkow recognized that the earliest lesions appeared in the arch near the orifices of branch points and then moved caudally. [From ref. (20).]

Were his findings not widely known? Was that the reason they were not followed up more aggressively? Not at all. Anitschkow published in German in the most respected and widely read journals of the time, and he published a long series of papers on the subject over the next few years. As mentioned above, a number of laboratories did try to reproduce his results, so many investigators were aware of his work. In 1933, Anitschkow published in English an extensive review of the work of his laboratory and that of others in *Arteriosclerosis,* a widely quoted collection of authoritative reviews edited by Cowdry (20). So again, at least the community of scholars interested in the pathogenesis of atherosclerosis was aware, or at least should have been aware, of his work.

Aside from these specific reasons for why Anitschkow's findings may have been ignored, there is another, and possibly the most important. His findings were inconsistent with the prevailing view of atherosclerosis. It was generally accepted to be an inevitable accompaniment of aging (the "senescence hypothesis"); it was a chronic, slowly progressive deterioration developing over decades. How could one possibly expect, the argument went, to mimic such a disease by feeding cholesterol to young rabbits for just weeks or months? It seemed totally implausible. There were lesions to be sure, but they could hardly be considered a model for human atherosclerosis.

In retrospect, Anitschkow's body of work showed clearly and convincingly that hypercholesterolemia in rabbits was a sufficient cause of atherosclerosis. Of course, it did not necessarily follow that cholesterol, either in the diet or in the blood, was also an important factor in human atherosclerosis. That conclusion would have to await studies showing that hypercholesterolemia in humans was indeed associated with atherosclerosis and, ultimately, clinical trials to establish causality. However, Anitschkow's work should have galvanized the scientific community and started a more serious approach to this major human disease problem many years ago.

Here was a classic example of how rigid preconceived ideas can stand in the way of scientific progress. An opportunity was lost. Why did no one ask the (now) obvious questions: How is the cholesterol carried in the rabbit blood? How does it get into the arterial wall? Which white blood cells are entering the artery wall and taking up huge amounts of cholesterol? Does the diet, especially the fat and cholesterol in it, increase blood cholesterol in humans? Answers would come, but only about 40 years later. The main point is that those questions were apparently not even asked at the time.

Anitschkow's work was done in St. Petersburg/Leningrad between 1912 and 1964, first at the Military Medical Academy and later at the Institute of Experimental Medicine. In 1962, just two years before his death, Anitschkow established a new Laboratory of Lipid Metabolism within the institute and named Dr. Anatoly N. Klimov to head it. A few years later, Klimov and collaborators (L. P. Rodionova and L. G. Petrova-Maslakova) carried out an experiment of heroic proportions to show definitively that the atherosclerosis in Anitschkow's rabbits was a direct result of the increased plasma lipoproteins and not some other indirect consequence of the cholesterol-rich diet. They isolated serum from cholesterol-fed rabbits, removed the chylomicrons, and gave it intravenously to chow-fed recipients. Over a 5- to 7-month period, the recipients received 14–25 g of cholesterol intravenously and developed significant arterial lesions. So blood cholesterol, mainly in LDL and VLDL, was the immediate and sufficient cause of the atherosclerosis (20). There is something satisfying in the continuity of focus on atherosclerosis at this institute, where Klimov remained as head of the Department of Biochemistry until his recent retirement.

WHAT LED ANITSCHKOW TO FEED RABBITS CHOLESTEROL?

Before leaving the Anitschkow story, it is of interest to ask just what inspired those classic studies. Like so many breakthroughs in science, it develops that Anitschkow's

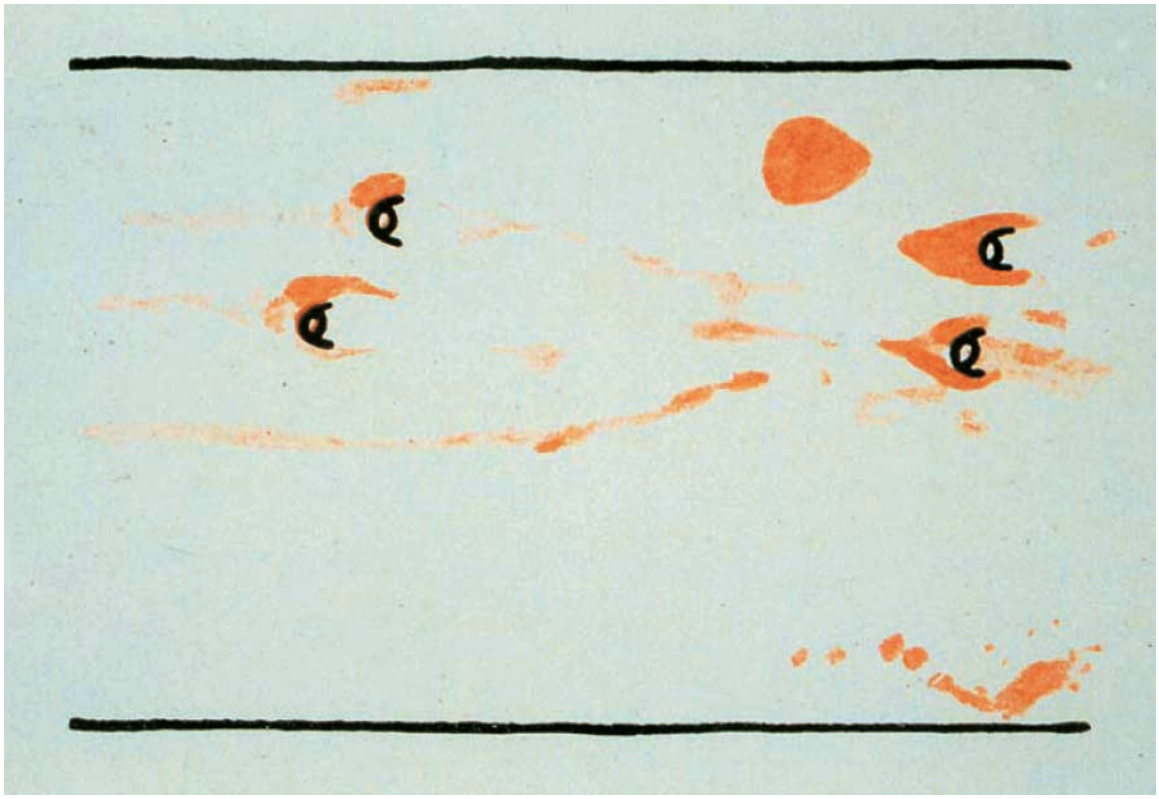

Fig. 4. Thoracic aorta from the same rabbit shown in Fig. 3. Anitschkow minutely described the appearance of "small yellowish spots of a triangular or semilunar shape, situated close below the orifices of [intercostal arteries]." He noted, in the abdominal aorta, "spur shaped thickenings . . . below the orifices, which probably serve the formation or regulation of various currents in the blood stream" that are "principally affected by the atherosclerotic changes." He clearly stated the hemodynamic hypothesis for specific localization of lesions. [From ref. (20).]

discovery was serendipitous. Many have probably assumed that Anitschkow was led to do his experiment by the 1910 Windaus paper (23) reporting that the aortas of patients with atherosclerosis contained much higher concentrations of cholesterol than did normal aortas. That is not the case. The rationale for Anitschkow's studies, as pointed out by Hoeg and Klimov (24), actually came from a 1909 paper by Ignatowski (25), another young experimental pathologist working at the Military Medical Academy in St. Petersburg. Ignatowski was pursuing an hypothesis put forward some years earlier by the Nobel Prize-winning microbiologist I. Metschnikow. Metschnikow proposed that an excess of protein in the diet was potentially toxic and somehow accelerated the aging process. So Ignatowski decided to feed rabbits a protein-rich diet and look for signs of such toxicity. He fed his rabbits large amounts of meat, eggs, and milk. These protein-rich diets were indeed toxic in young rabbits, affecting liver and adrenals, but in adult rabbits the major effect was the development of striking arterial lesions resembling those of human atherosclerosis (25). Because atherosclerosis was considered one of the hallmarks of aging, Ignatowski considered that his findings had confirmed Metschnikow's "protein toxicity" hypothesis. It remained for Anitschkow and Chalotow (18) to progressively narrow things down and show that the vascular damage could be induced by simply feeding cholesterol purified from egg yolks without the eggs or milk or meat (i.e., without the protein). Another instance of an unpleasant fact destroying a beautiful hypothesis. However, in this instance, the new findings generated a very valuable new hypothesis, the lipid hypothesis of atherogenesis.

THE BIRTH OF THE LIPOPROTEINS

During the first two decades of the 20th century, a number of investigators drew attention to the fact that the considerable amounts of lipid in serum must be present either in some sort of emulsion or in association with proteins. However, the first definitive studies on the nature of the plasma lipoproteins were reported by Macheboeuf and colleagues beginning in 1929 (26). Over the next 10 or 20 years, they succeeded in purifying and carefully characterizing α-lipoprotein from horse serum and showed that the lipid-free protein remained soluble. During World War II, Cohn et al. (27) and Oncley, Scatchard, and Brown (28) at Harvard developed elaborate large-scale methods for fractionating human serum to provide materials useful in treating the wounded. In the course of those systematic studies, they found that the lipids of se-

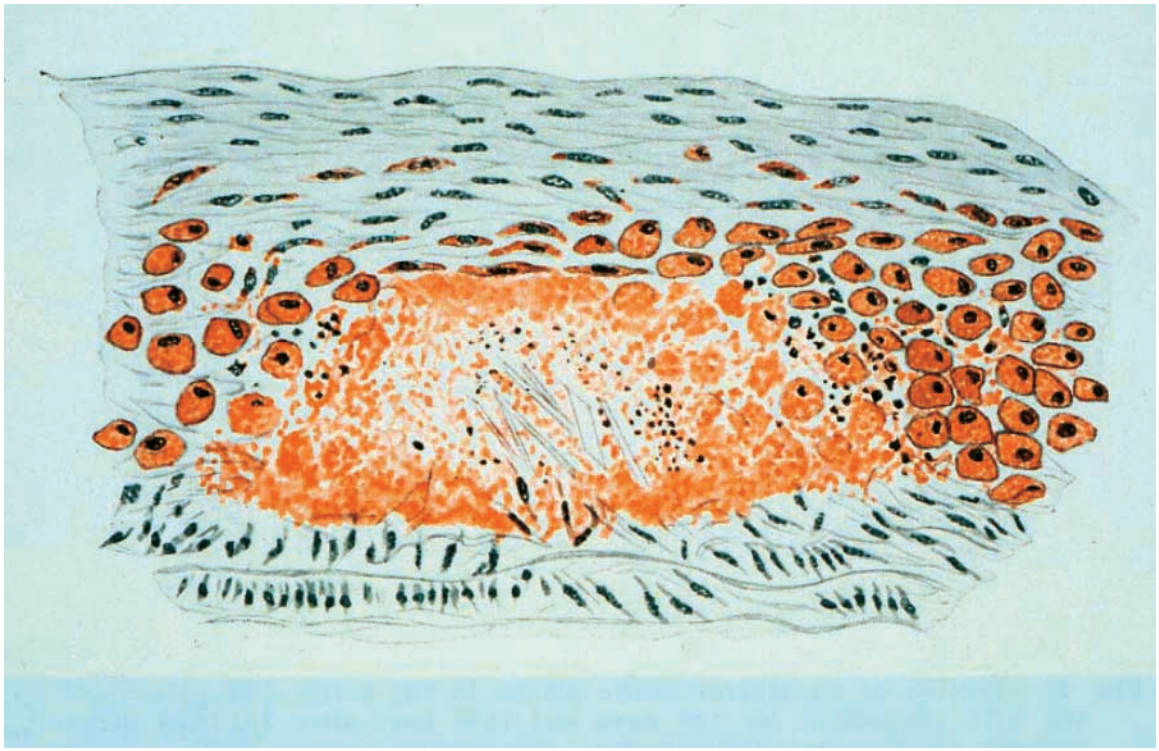

Fig. 5. Advanced plaque in the aorta of a rabbit fed cholesterol for 124 days and then put back on a chow diet for 101 days before killing. Anitschkow calls attention to the central necrotic lipid core containing needle-like crystals of cholesterol, scattered calcium granules, groups of foam cells on either side of the core, and a fibrous cap overlying the lesion. [From ref. (20).]

rum were concentrated in two major fractions having α_1- and β-mobility, respectively.

At the time, nothing was known about their origin, their fate, or their biological significance. Then, in 1951, Russ, Eder, and Barr (29) at the New York Hospital-Cornell Medical Center, using the methods developed in the Cohn/Oncley laboratory, made the important finding that women before menopause had consistently higher blood levels of the α-lipoprotein than did men. They speculated that this difference might be related to the lower incidence of coronary artery disease in premenopausal women, and time has certainly proved them right. Their 1951 paper was possibly the first demonstration that different lipoprotein classes might have distinct biological functions. It also was the first suggestion of a linkage between specific lipoprotein patterns and the risk of coronary heart disease. However, their speculation was at that time based only on a correlation and did not establish a causal relationship. Today, that causal relationship has been established beyond a doubt.

HOW MANY DIFFERENT LIPOPROTEINS ARE THERE AND WHAT DO THEY DO?

John W. Gofman was not the first to try to characterize the full spectrum of lipoproteins in the blood, but he was the first to do so successfully (**Fig. 7**). Gofman is a prime example of the unusual man who straddles two fields and as a result is able to see novel ways of applying methods and ideas from one field to the other. He started medical school at Case Western University in Cleveland, but before finishing he went on to the University of California, Berkeley. There, he came under the influence of two giants, both Nobel Prize winners: Ernest O. Lawrence, inventor of the cyclotron, and Glenn T. Seaborg, who created 10 transuranium elements using that cyclotron. Gofman was quickly swept up in the excitement of the Lawrence laboratory as part of the atomic bomb team. He stayed on and earned a Ph.D. in physics under Seaborg's direction and then entered medical school at the University of California, San Francisco across the bay.

Gofman had always been certain he wanted to do biomedical research, and he leaned toward research on cardiovascular disease. He was familiar with Anitschkow's work, and he, unlike most others at the time, took it very seriously. That work, together with the genetic, biochemical, and epidemiologic evidence available (albeit limited), convinced Gofman that blood cholesterol, and the dietary determinants of blood cholesterol, was centrally important in atherosclerosis. His level of conviction is attested to by the introduction he wrote for a book his wife, Dr. Helen F. Gofman, published in 1951 with several others at Berkeley (30). This was possibly the first low-fat, low-cholesterol "diet-heart" cookbook ever published. Clearly, Gofman accepted Anitschkow's dictum that cholesterol in the blood somehow played a causative role in atherosclerosis. At the same time, he recognized that almost nothing

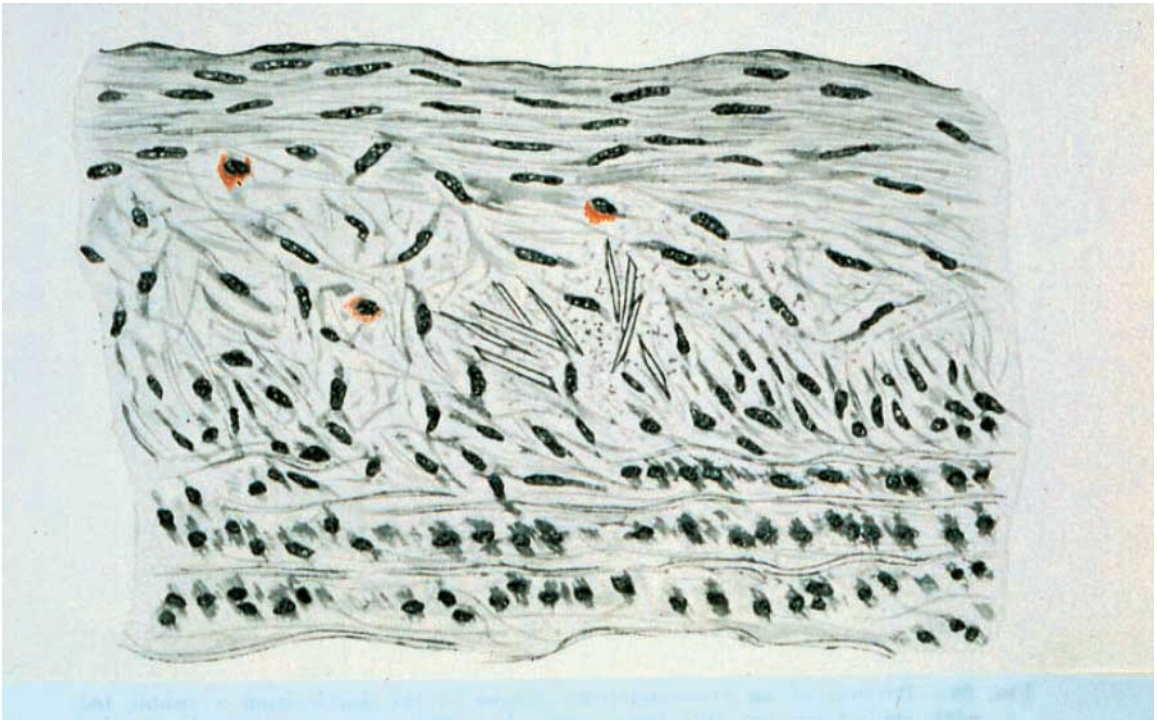

Fig. 6. Atherosclerotic plaque from the aorta of a rabbit fed cholesterol for 106 days (i.e., approximately the same as the rabbit shown in Fig. 5) but then returned to a chow diet for 785 days (more than 2 years!) before killing. Anitschkow describes it as follows: "The lipoidal masses have disappeared; only a few cholesterin [cholesterol] crystals and lipoid-containing wandering-cells are still present." He adds that the surface is "fibrous and dense." He recognized that even late lesions were at least partially reversible (i.e., at least the lipid from them). [From ref. (20).]

was known about the chemical form or forms in which that blood cholesterol was carried.

Gofman's unique background enabled him to open up new territory. Because of his strong background in physics and chemistry, he could see the potential power of a then new and highly sophisticated technique: analytic ultracentrifugation. The technique had been developed in Scandinavia by Svedberg and proved to be invaluable for characterizing proteins and measuring their molecular sizes and relative concentrations in mixtures. Gofman wanted to see if it could be used to further characterize the lipoproteins in human serum. At the time, there were only a few such instruments in the entire world. Moreover, Pedersen, the world's expert in Sweden, had already tried to study the proteins in whole serum but had encountered an artifact he could not explain (31). All serum samples contained what he called the "X-protein," present in varying concentrations from sample to sample and, much worse, seeming to change in concentration during the analytical centrifuge run. Pedersen had good evidence, from his own work and that of others, that the X-protein must be a lipoprotein (31–33). He had shown that it floated to the top of the centrifuge tube if salt was first added to the serum, and he knew that it was rich in associated phosphatides. After extraction of the lipids (31, 32) or after treatment with lecithinase (33), the X-protein disappeared. Pedersen had even estimated its molecular weight at 1.9×10^6, an excellent estimate for LDL! However, he did not recognize the reasons for the apparent changes in concentration during analysis, still thinking that they must reflect some reversible aggregation of X-protein with other plasma proteins. From the Schlieren pattern, he estimated that protein X would have to account for as much as 30% of total serum proteins, which seemed implausible! He had given up trying to work with whole serum.

The true explanation of the X-protein artifact was discovered by Gofman with his collaborators, Frank T. Lindgren and Harold Elliot, and published in 1949 (34). They showed very elegantly that the apparent artifact was attributable to the presence of the very thing they wanted to study: lipoproteins. What was happening was that LDL, less dense than albumin but dense enough to sediment at the density of serum, was also moving down the tube but more slowly. As the concentration of albumin built up, the combined background density of serum and that of the albumin at the boundary now exceeded that of LDL. Consequently, LDL at that point in the tube ceased sedimenting and tended to migrate toward the top of the tube. The Gofman team showed unequivocally that LDL and other lipoproteins were the basis for the X-protein artifact, When they simply added salt to the serum so that the lipoproteins floated instead of sedimenting, they got highly reproducible results. Instead of trying to analyze whole serum, they first floated all of the lipoproteins, thereby concentrating them and eliminating the X-protein artifact. They went on to devise accurate and reproducible ways to

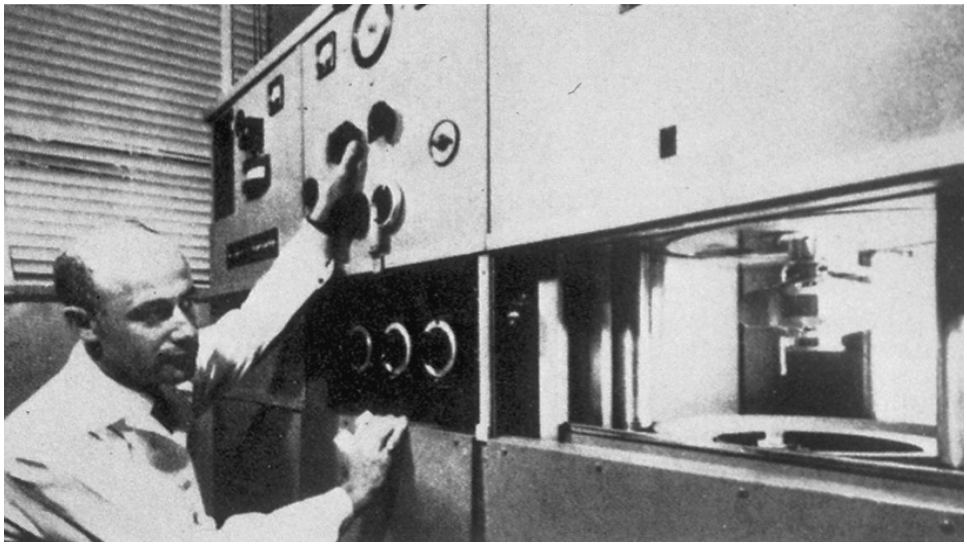

Fig. 7. The young John Gofman getting ready to fire up his analytical ultracentrifuge for a lipoprotein separation at the Donner laboratory (circa 1948). This was only the second instrument of its kind; it was designed by Ed Pickels and built by the Specialized Instruments Corporation. Reproduced with permission of Dr. John W. Gofman.

separate the lipoproteins in the plasma into subclasses and to measure their concentrations reliably. This was the breakthrough they had been looking for, and now they were off and running with a view to studying the relationship between coronary heart disease and not just total cholesterol, but also the concentrations of the different classes of lipoproteins that carried that cholesterol.

In a 5- or 6-year period beginning in 1949, Gofman and his collaborators turned out a prodigious amount of new information about the lipoproteins in human plasma, their metabolism, and their correlation with atherosclerosis. Their basic "credo" was that it mattered a great deal in which lipoprotein fractions blood cholesterol was carried, and in 1950 they presented preliminary data on a limited number of patients suggesting that the S_f 10–20 fraction was particularly proatherogenic (35). This fraction corresponds closely to intermediate density lipoproteins, small remnants of which do indeed seem to be strongly proatherogenic. Later, they would propose a formula to weight the different subclasses according to their presumed atherogenicity, a so-called "atherogenic index" (36). Whether or not this index was more predictive than total blood cholesterol became a highly controversial issue, as discussed below. However, this aside, Gofman's laboratory made a number of important observations and, most importantly, they started people thinking about the serum lipoproteins and looking into their relationship to atherosclerosis. For example, they identified a small group of patients having characteristic skin xanthomas (xanthoma tuberosum) and a unique lipoprotein pattern. They suggested that this was a distinct disease entity, one carrying a very high risk of coronary heart disease. What they described is what we now know as dysbetalipoproteinemia, which is indeed the result of a specific mutation in apolipoprotein E. Gofman and his group also did a nice pioneering study of the postheparin clearing phenomenon. They used the analytical ultracentrifuge to demonstrate that the clearing was accompanied by a progressive decrease in the size of the larger lipoproteins to smaller, less buoyant forms, a shift that decreases light scattering (37). That work anticipated the discovery that the whole process was enzymatically triggered by lipoprotein lipase, a discovery made soon thereafter by C. B. Anfinsen, E. Korn, and colleagues at the National Heart Institute, as will be discussed in more detail in Part II (38).

As described above, Gofman's early work showed a good correlation between lipoprotein increase and risk of heart attack, but the sample sizes in these preliminary studies were small. Still, the data suggested that the different classes of lipoproteins should be weighted differently in estimating the risk of coronary heart disease using the atherogenic index (36). Gofman proposed that this index, or a similar profile of the kinds of lipoproteins that were increased and their concentrations, should predict coronary risk better than just the total cholesterol level. What was needed to clinch the case was a large prospective study. Why didn't Gofman or others interested in the heart disease problem just plow ahead and do such a study? To do so would have required making analytical centrifuge runs on hundreds of individuals. There were at the time only two such instruments in the whole country. Moreover, they were extraordinarily expensive and technically difficult to operate. Finally, most research workers were quite skeptical about the value of what Gofman was doing. Nevertheless, his preliminary results were so impressive that the NIH eventually decided to fund a large cooperative project to test Gofman's ideas in a prospective manner. In a personal communication, Gofman tells an interesting story about how that study got off the ground.

Gofman had already applied to the NIH for a large grant that would let them move ahead more rapidly. To

run the requisite number of samples in a reasonable length of time meant buying an additional analytic centrifuge and expanding the laboratory staff, so his request was for $70,000 per year (equivalent to more than $500,000 in current dollars). The application was turned down and Gofman was distraught. Without additional funding, he could not critically test the generality of his hypothesis. At about the same time his grant was turned down, he was at invited by an old friend, Lawrence Spivak, to write an article on his lipoprotein work for the *American Mercury* magazine, which Spivak edited at the time. (Later, Spivak would become more widely known for launching *Meet the Press* with Martha Rountree.)

When Gofman told Spivak what had happened, Spivak promptly placed a telephone call to Mary Lasker to ask for her advice. Lasker was a force to be dealt with. She was a tireless supporter of biomedical research. She and her husband gave generously of their own money, but, even more importantly, she directly lobbied the Congress on behalf of NIH. She is credited with playing a role second in importance only to that of Dr. James A. Shannon for building the research budget of NIH at an astonishing rate during those early postwar years. Mary Lasker immediately made an appointment to see Gofman at her Beekman Place apartment in New York. She was taken with Gofman and impressed by the promise of what he was doing, and she, in turn, made a key telephone call. She called T. Duckett Jones, professor of medicine at Harvard, and asked him to please come down to New York and meet Gofman. Jones was there the very next day; he listened to Gofman's story and said, "John, you need help. You're not going to get that grant unless you get help. What I think you need to do is to get two or three additional laboratories to agree to join you as collaborators in this study in order to make it saleable. We'll just have to call in some other people."

The upshot of all this was that three other research centers (the Cleveland Clinic, the University of Pittsburgh, and the Harvard School of Public Health) joined with Gofman and proposed to the NIH a cooperative study using the Gofman type of analysis. So, the NIH, having turned down Gofman's request for $70,000, was now investing $280,000 in a four-center project. The decision may have involved some bending of standard operating procedure, but it turned out to have been a sound one. Over a period of 3 years, the investigators analyzed the lipoproteins of almost 5,000 men aged 40–59 years who were clinically normal at the time they were first studied. These men were carefully followed over a 3-year period during which there were 82 cardiac events (myocardial infarction or development of new angina pectoris) classified as definite or probable. The issue was whether the pattern of lipoproteins determined with the analytical centrifuge would or would not be a better predictor of those who were going to have an event than simply the measurement of total cholesterol in the blood. After the study had already begun, Gofman became aware of a technical glitch in his methodology but also figured out a way to correct it. However, by that time, a large number of samples had already been used up or there was not enough left to reanalyze. As a result, the final report from three of the four participating laboratories had to be based on the original method only, whereas the results from the Donner laboratory were corrected and reported in both the original form and the revised, more accurate form. The final report, published in 1956, contained two formal Discussion sections, one representing the views of the Donner laboratory and the other representing the views of the other three centers, an unfortunate schism (39). However, the results contained important lessons. It was clear that either the total cholesterol level or the lipoprotein pattern identified those at high coronary heart disease risk. The lipoprotein pattern was not necessarily superior to the measure of total cholesterol level but it was just as good.

The original protocol for the cooperative study defined "definite events" to include angina pectoris, a subjective finding. In Gofman's dissenting report, he analyzed the data with and without angina pectoris included and found that the predictive value of lipoprotein analysis (both total cholesterol and ultracentrifuge analysis) was much greater when this subjective end point was eliminated. At the time, there was quite a fuss about the Gofman dissenting report, and feelings ran high in some quarters. What was lost sight of at the time, and even in retrospect, is that in 1956 these investigators had provided important additional evidence that cholesterol-carrying molecules in the blood predicted the risk of heart disease. Later studies would show that different lipoprotein fractions do indeed have different degrees of relevance to atherosclerosis (i.e., the phenotype does count). Today, LDL is recognized as the most atherogenic of the lipoproteins, which agrees with Gofman's findings. Later studies also showed that VLDL is less predictive than is LDL but correlates positively with risk. So, Gofman was basically correct, but unfortunately the data from the cooperative study by itself did not make the case. The analytic ultracentrifuge gave way to the preparative ultracentrifuge in lipoprotein research (40) and to paper electrophoresis in clinical research (41). Gofman himself began to turn his attention more and more to the issue of radiation hazards, but the Donner laboratory under Frank T. Lindgren, Alex V. Nichols, and their collaborators continued to exploit the ultracentrifuge as a highly valuable research tool.

The impact of Gofman's work on the field was of great and lasting importance. He opened the window on the complexity of the lipoproteins and started people thinking about what they do, how they are metabolized, and how they lead to atherosclerosis. The next two decades would see an explosive increase in research on the plasma lipoproteins and their relationship to atherosclerosis. Anitschkow and Gofman played major roles in sparking that explosion. [To be continued.]

The author acknowledges with thanks the help of Dr. Anatoli Klimov in putting together the Anitschkow story and of Dr. John W. Gofman for agreeing to be interviewed and for providing background documents. Thanks also to Drs. Richard J. Havel, John W. Gofman, John Kane, and Joseph L. Witztum for valuable discussions and comments.

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