

Thematic Review Series: Living History of Lipids

In celebration of the 100th anniversary of the lipid hypothesis of atherosclerosis

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Abstract This year marks the 100th anniversary of the publication of Anitschkow's classic paper proposing the central role of hypercholesterolemia in atherogenesis. We at the *Journal of Lipid Research* take this occasion to acknowledge the debt we all owe to Anitschkow and his colleagues for getting us on the right track. As discussed below in detail, his contributions were insightful and went well beyond simply pinpointing hypercholesterolemia as a major etiologic factor. Anitschkow's work led him to define most of the key elements in the initiation and evolution of lesions in animal models of atherogenesis.—Steinberg, D. In celebration of the 100th anniversary of the lipid hypothesis of atherosclerosis. *J. Lipid Res.* 2013. 54: 2946–2949.

It is difficult to believe that the key role of cholesterol in the pathogenesis of atherosclerosis was proposed 100 years ago! Yes, in 1913, Nikolai N. Anitschkow, a young Russian experimental pathologist in Saint Petersburg, reported that simply feeding rabbits a high-cholesterol diet produced arterial lesions that closely resembled those of human atherosclerosis (1–3). He and one of his medical students, S. Chalатов, purified cholesterol from egg yolks, dissolved it in sunflower oil, and fed it to normal rabbits. Their blood cholesterol levels rose sharply, and within weeks their arteries began to show raised yellow lesions rich in “lipoids.” They exhibited structural features very much like those seen in the human disease. Anitschkow summarized the results of his pioneering work on the rabbit model of atherosclerosis in this way:

The blood of such animals exhibits an enormous increase in cholesterin [cholesterol] content, which in some cases amounts to several times the normal quantity. It may therefore be regarded as certain that in these experimental animals large quantities of the ingested cholesterin are absorbed, and that the accumulations of this substance in the tissues can only be interpreted as deposits of lipoids circulating in large quantities in the humors of the body. (3)

By 1913 human atherosclerosis had been well described in the medical literature, but it was still considered to be an

inevitable accompaniment of the aging process, totally mysterious and certainly not treatable. At the time, one of the leading hypotheses as to its pathogenesis, favored by Metchnikow, was that it was the result of excessive intake of animal proteins. Experimental physiologists at the St. Petersburg Imperial Military Medical Academy, including Anitschkow, were seeking evidence to support Metchnikow's hypothesis. They first fed rabbits diets rich in milk, eggs, and meat, and indeed those rabbits displayed vascular lesions. They went on to determine whether any particular kinds of protein were involved. By a process of progressive elimination they found that whole eggs or egg yolks alone would do the trick, but egg whites alone, even in large amounts, did nothing! Finally, Anitschkow and Chalатов showed that cholesterol, extracted from the egg yolks, purified, and dissolved in vegetable oil, could by itself duplicate the results without any added protein at all. In their classic 1913 paper they wrote:

The main thrust of our investigations is that... it becomes totally clear why only certain nutrients, for example egg yolks or brain, can evoke specific changes in the organism. Since the same changes can be observed by feeding pure cholesterol, there remains no doubt that it is precisely this substance that is laid down in the organism as liquid-crystal droplets and evokes extraordinarily damaging effects in various organs. (1)

An English translation of this article was published in *Arteriosclerosis* in 1983 (2). Thus the protein toxicity theory of aging and atherosclerosis, like many other beautiful theories, had been slain by a few ugly facts and the lipid hypothesis (\equiv the cholesterol hypothesis) was born (**Fig. 1**).

THE SCOPE OF ANITSCHKOW'S CONTRIBUTIONS

If Anitschkow had not gone beyond these early observations he might not have left much of a mark. Obviously a

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Originalmitteilungen.

Nachdruck verboten.

Ueber experimentelle Cholesterinsteatose
und ihre Bedeutung für die Entstehung einiger
pathologischer Prozesse.

Von Dr. med. N. Anitschkow und Dr. S. Chalатов.
(Aus dem pathologisch-anatomischen Institut der Kaiserlichen militär-
medizinischen Akademie zu St. Petersburg.)

Fig. 1. Title page of the classic 1913 Anitschkow and Chalатов paper.

great deal remained to be done before the cholesterol-fed rabbit could be accepted as a suitable model for the human disease. But Anitschkow did go much farther. He was a careful experimentalist with a keen eye for detail, and over the next two decades he and his group in St. Petersburg carefully documented most of the salient features of rabbit atherosclerosis. In 1933 he presented a comprehensive review of his own work and that of other laboratories in a volume edited by Cowdry (3). In the 20 years following his first paper, he presented data on the following aspects of the disease.

Foam cells

In the earliest lesions—the fatty streaks—most of the lipid is found inside cells in multiple, small lipid droplets. Because lipids are extracted during the routine preparation of tissue samples, the multiple lipid droplets are seen as empty vacuoles; hence, the designation “foam cells.”

Cholesterol accumulation

In tissue sections the lipid droplets are birefringent. Anitschkow recognized birefringence as a characteristic property of liquid crystals of cholesterol esters.

White blood cell recruitment

The cholesterol-loaded foam cells are white blood cells that have infiltrated the artery wall. Thus, Anitschkow anticipated that inflammation might play a role in lesion development.

Structurally intact endothelium

The monolayer of endothelial cells over the lesions appears to be intact, indicating that the invading blood cells must have penetrated between the endothelial cells. Thus, endothelial denudation, while it clearly did occur at a later time, was not a necessary antecedent to lesion formation.

Nonrandom anatomic distribution of lesions

There is a characteristic, reproducible pattern of lesion distribution. They occur most commonly and most severely at arterial branch points. Anitschkow correctly surmised that this localization was determined by hemodynamic factors.

Conversion of fatty streaks to fibrous plaques

Over long periods of cholesterol feeding (months) there is ultimately deposition of connective tissue (conversion of

the fatty streak to the fibrous plaque) and development of a fibrous cap. (In the human disease, it is rupture of this fibrous cap that precipitates thrombosis and myocardial infarction; neither the rabbit model nor other animal models reproduce this terminal thrombotic event with any regularity.)

Reversibility

Early lesions are partially reversible but the reversal is slow; late lesions resolve even more slowly. Most, but not all, of the lipid can be mobilized from advanced lesions, leaving behind the fibrous cap and a few cholesterol crystals.

Severity of lesions proportional to increase in blood cholesterol level

The extent and severity of lesions is proportional to the degree of blood cholesterol elevation and the duration of exposure to it. Anitschkow was well aware that it was the level of blood cholesterol that determined the size and extent of lesions, not necessarily the amount of cholesterol ingested.

High blood cholesterol necessary but not always sufficient (notion of multicausality)

While blood cholesterol level is critically important, other factors can and do play a significant part in atherogenesis. Anitschkow's dictum “No atherosclerosis without cholesterol” has often been cited as showing that he was unaware of the multifactorial nature of the disease. However, his 1933 review (3) gives the lie to this. There he sums up as follows: “The views here set forth concerning the etiology of atherosclerosis constitute what I have called the ‘combination theory’ of its origin.” So, it should be clear that he was fully aware that the degree of atherosclerosis, while perhaps most evidently dependent on the degree of blood cholesterol elevation, could be significantly affected by other factors, such as blood pressure, toxic substances, and local arterial changes. In his rabbit model, however, no such additional insults or injuries were needed; hypercholesterolemia was a sufficient cause. The correctness of this conclusion was most dramatically underscored by Watanabe's discovery in 1980 of a strain of rabbits that have blood cholesterol levels around 600 mg/dl, like that of the cholesterol-fed rabbits, and uniformly develop atherosclerosis on a regular chow diet. These rabbits have a mutation of the LDL receptor gene identical to that found in some humans with familial hypercholesterolemia. Thus, the defect in LDL receptor function leads to a sharp reduction in rate of LDL removal from plasma and the consequent sharp increase in LDL levels. In these cases, hypercholesterolemia is itself a sufficient cause of atherosclerosis. However, as Anitschkow recognized, the rate of progression of lesions at any given level of LDL can be importantly slowed or accelerated by other factors, such as hypertension or disorders of the immune system.

It is quite remarkable how well Anitschkow's description of atherogenesis has stood the test of time. While there have been many advances at the level of biochemistry, cell

biology, and molecular biology, the basic pathogenesis in animals as he described it 100 years ago requires little or no amendment. However, building the case for the importance of hypercholesterolemia in human atherosclerosis was an uphill battle. General acceptance would have to wait for over 60 years when, in 1984, the National Heart Institute completed the first large-scale, randomized, double-blinded clinical trial showing that lowering blood cholesterol significantly lowered the risk of myocardial infarction (4, 5). That was the landmark seven-year Coronary Primary Prevention Trial using cholestyramine, a bile acid sequestrant. Taken together with the many other lines of evidence implicating blood cholesterol as causative, it was the basis for an NIH Consensus Conference (6) and formulation of national guidelines for management of elevated blood cholesterol levels (7) (Fig. 2).

WHY WASN'T ANITSCHKOW'S LEAD FOLLOWED UP?

Some laboratories did try to confirm Anitschkow's findings. Bailey at Stanford, using rabbits and guinea pigs, quickly confirmed Anitschkow's findings (8, 9). Most investigators, however, instead of using rabbits, used the laboratory animals they were more familiar with—rats or dogs. Cholesterol feeding in these species failed to induce lesions. Understandably, these investigators concluded that Anitschkow's results must reflect some peculiarity of the rabbit. After all, they said, 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 the human disease.

What was not appreciated in Anitschkow's day was the fact that rats and dogs, unlike rabbits, are very efficient in converting cholesterol to bile acids. Consequently, even on very high intakes of dietary cholesterol, the blood cholesterol in these species does not rise appreciably. Steiner and Kendall, 33 years later, would show that first inhibiting thyroid function in dogs with thiouracil (which decreases the LDL receptor number) and then feeding them cholesterol increases blood cholesterol and induces lesions (10). So here was one reason Anitschkow's work was



Fig. 2. Anitschkow shortly after his retirement.

not taken seriously: failure to recognize the two-step nature of what was going on—feeding of cholesterol, followed by elevation of blood cholesterol levels, followed by atherogenesis. Only if the second step kicks in does one get atherosclerosis.

Another reason Anitschkow's findings were not taken seriously is that the blood cholesterol levels in his rabbits were extraordinarily high—500 to 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 elevations of cholesterol levels in rabbits were sufficient to induce lesions. It just took longer.

Were his findings not widely known? Was that the reason they were not followed up more aggressively? Not at all. He did not publish in Russian but in German and in the most respected and widely read journals of the time. Also, as discussed above, in 1933 Anitschkow published, in English, an extensive review of the work of his laboratory (3). So at least the community of scholars interested in the pathogenesis of atherosclerosis should have been aware of his work.

There is another and possibly more important reason for the indifferent response of the scientific community. Anitschkow's findings ran counter to the prevailing view of atherosclerosis. Atherosclerosis 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 to mimic such a disease—the argument went—by feeding cholesterol to young rabbits for just weeks or months? It seemed totally implausible. 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 that relationship as a causal one. Interested readers will find a history of the sometimes quite violent controversies that surrounded the lipid hypothesis and how it finally became accepted in *The Cholesterol Wars* (11). Anitschkow's work should have galvanized the scientific community and encouraged innovative approaches to this major human disease problem. But nothing happened. Here was a classic example of how rigid, preconceived ideas sometimes stand in the way of scientific progress. 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 about 40 years later. Those kinds of questions were apparently not even asked before World War II. Finally, in the 1950s, the metabolic studies of John W. Gofman (12) and Lawrence W. Kinsell (13), and the classical epidemiologic studies begun

by Thomas R. Dawber and coworkers in Framingham, MA, (14) sparked a renewed interest in the cholesterol-heart disease connection.

Should Anitschkow's contributions have earned him a Nobel Prize? I have discussed this question elsewhere and concluded that the importance of his discovery most probably would have been recognized by the Prize—if only the timing had been different (15). The main problem was that he was too far ahead of his times. Anitschkow was born in 1885 and wrote his classic paper in 1913. Yet the validity of the lipid hypothesis did not become generally accepted until 1984. (Believe it or not, there are still a few pockets of stout resistance!) We who have followed in Anitschkow's footsteps salute him on this 100th anniversary of his breakthrough paper. 

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