It often happens in the history of science that researchers, guided by quite different basic concepts and using different methods, obtain results which can be unexpectedly synthesized to produce interesting conclusions and which require us to view scientific questions in a new light. The very fact that we start with a diversity of viewpoints and observations strengthens our conclusions and gives them scientific validity.

In recent years such a fortunate situation is apparently starting to affect our thinking about cholesterin metabolism and storage. Data from different medical disciplines, particularly pathological histology, physiological chemistry, and clinical observations, are leading to a gratifying consensus about the importance of cholesterin in the animal body.

Only one aspect of this exceptionally interesting theory has not yet received sufficient attention, namely, the possibility of studying these problems using the methods of experimental pathology. These methods could help elucidate questions about the cholesterin content of various organs and could indicate the significance of these variations in the origin of many pathological processes.

Thanks to the work of several Russian researchers, we already have sufficient evidence to offer some suggestions from the standpoint of experimental pathology. Our own investigations, still in progress, are leading us toward what we hope may be a conclusive series of experiments.

As so often seems to happen, the authors of the first relevant papers began from a point that initially had no apparent relationship to the theory of cholesterin metabolism. In 1908 Ignatowski published the results of his experiments, which had been undertaken to study the effect of feeding animal products, particularly egg white, on the rabbit. The general experimental plan was to supplement the usual vegetarian diet of the rabbit with various types of foods of animal origin, specifically, ox meat, chicken eggs, and milk. The animals were given this food for 21 to 196 days.

In these experiments it was demonstrated that the aortic walls and the liver suffered the greatest changes in response to nutrients from animal sources. Further, a noticeable hypertrophy of the adrenal glands and parenchymatous changes of the kidneys were identified in the experimental animals. Ignatowski believed he could explain all these changes by the harmful effect of animal protein (egg white) in the food.

Ignatowski's results were confirmed in the next year by Starokadomski, who observed extraordinarily marked changes in the aortas of rabbits fed chicken egg yolks blended in cow's milk. He also presented a comprehensive description of the microscopic aspects of these changes. However, one could still not judge from these investigations whether it was specifically the egg white of the animal food, as Ignatowski had assumed, that elicited the changes described above.
Although d'Amato and Simon and Garnier had earlier emphasized the importance of animal egg white and its products in the origin of some forms of illness, it was still not possible to draw an analogy between the results of their experiments and those of Ignatowski and Starokadomski. Lubarsch and his co-workers fed rabbits liver and adrenal substances, and their results appeared to disagree completely with the results of the Russian authors. However, it is clear from Lubarsch's article that his experiments were almost exclusively concerned with necrotic changes in the media of small arteries. These changes are accompanied only by a secondary unessential thickening of the intima in which fat deposition, so characteristic of human atherosclerosis, is absent.

In contrast, in Starokadomski's experiments the major changes were always localized in the intima and were characterized by a rich infiltration of fatty substances. Furthermore, in the experiments of both Starokadomski and Ignatowski, prominent changes were found in the aorta, as compared to changes in only the small arteries in Lubarsch's study.

This difference in results was all the more striking because the fundamental ideas behind both series of experiments (Lubarsch on the one hand, Ignatowski on the other) appear to be alike: both researchers wanted to follow changes in rabbits forced to consume food of animal origin.

A review of these two sets of experiments which yielded such different results shows that the animal nutrient materials used by the two authors had different compositions. In fact, the experiments of Ignatowski and Starokadomski already suggest the hypothesis that egg yolk may be the nutrient that evokes significant aortic changes with intimal thickening and fatty infiltration. Egg yolk was not used in the experiments described by Lubarsch; in his experiments, liver and adrenal substances as well as some types of meat formed the sole nourishment for the experimental animals.

However, from Starokadomski's experiments one still could not be certain of the interpretation stated above; Starokadomski's feeding experiments were always carried out on the same animals that Klotz was using in his suspension experiments, and these latter experiments could have conceivably caused a series of changes in the aorta. Thus in 1910, Stuckey, at the suggestion of Professor Moissejoff, undertook a new series of exhaustive experiments which not only settled the questions that had been raised but also served as the starting point of all succeeding investigations. In Stuckey's experiments various types of animal products were tested with regard to their capacity to elicit aortic changes in rabbits. Specifically, these foods were muscle fluid, chicken egg white, egg yolk, and milk. While muscle fluid, egg white, and milk had very little harmful effect on the aortic walls, the rabbits fed egg yolk manifested an enormous intimal hypertrophy and infiltration of fatty substances.

Because these changes corresponded completely to those found by Ignatowski and by Starokadomski, both of whom used foods containing egg yolk, it was suspected that specifically the egg yolk causes the harmful effects. Through Stuckey's experiment, Saltykow's outspoken opinion was discredited. Saltykow claimed that in all relevant experiments cow's milk is the harmful foodstuff, because the egg yolk in these experiments was often blended with cow's milk. Stuckey did not, however, succeed in producing changes in the rabbit aorta with a mixture of milk and muscle fluid, which certainly would be expected if cow's milk could independently elicit the same changes. Furthermore, Wesselkin, who had fed rabbits only cow's milk in his control experiments, could find no changes in these animals.

The morphological changes described by Stuckey are quite different from the changes observed by Lubarsch after feeding the rabbits organ extracts; in fact, the changes noted by Stuckey most closely resemble human atherosclerosis. This exceptionally interesting finding motivated Stuckey to undertake a further experiment to analyze the problem; he investigated which of the substances actually present in egg yolk elicit such remarkable changes in the aortic walls when introduced into the rabbit. It was clear that the egg white contaminants of the chicken egg yolk preparations could play no role in this, because almost no changes of the aorta were seen in rabbits that were fed large amounts of egg white material (e.g., chicken egg white).

Because numerous fatty substances are dominant components of egg yolk, Stuckey used various animal and plant fats in his further experiments and tested their effect on the rabbit aorta. The results of these experiments were, however, completely negative. In only one group of experimental animals did he observe limited changes like those produced earlier with egg yolk; this was the group that was fed large amounts of brain.

This fact could be explained if the substances that produce the experimental "nutritional atheronecrosis," described by Ignatowski, Starokadomski, and Stuckey, occur in both egg yolk and brain. The ultimate solution to the question of the nature of these substances was first suspected when changes in other organs, especially the liver, were taken into consideration.

In 1911 and 1912 one of us (Chalatow) published the results of investigations which demonstrated that egg yolk as well as brain produce very characteristic changes in the livers of rabbits fed these substances. These changes consist of an extraordinarily rich infiltration of the liver parenchyma with fat-like substances that had the form of double refractive drops in fresh preparations. These drops show beautiful cross figures in polarized light and occasionally flow together to build cylindrical forms that resemble the so-called myelin-type figures. To characterize their physical properties, these double refractive drops could be considered liquid spherical crystals which,
The investigations cited above have shown that lipoid substances accumulate in enormous quantities in the liver parenchyma. In this organ these substances produce the distended appearance of degeneration of the liver cells with subsequent cirrhotic proliferation of connective tissue.

The nature of these lipoid substances could not, however, be confirmed with absolute certainty by means of conventional staining reactions. These reactions indicated only that a certain amount of phosphorus-containing lipoids is present in these liver deposits; this is demonstrated by a positive result in Dietrich's reaction. This particular method was originally thought to be specific for cholesterin, proved to be especially suited for identifying phosphorus-containing and other lipoids. (See the investigations of Kawamura.) Because all these methods of lipid determination lack specificity, Chalatow directed his attention to the physical properties of the lipoid materials in the liver.

After formalin fixation, the liquid spherical crystals present in the liver parenchyma were transformed into solid crystals whose morphologic properties and melting point were the same as the mixtures of cholesterin and fatty acids investigated by White.

From these findings it could be concluded that the materials in question probably belong to the cholesterol group. However, because the egg yolk contains other lipoids in large quantities, especially lecithin, one could not completely reject the hypothesis that to some extent this latter substance accumulates in the liver parenchyma and produces the changes described above. It was therefore of special importance to test lecithin by the method described; this was recently done by Wesselkin (I.c.). The results of his experiment were completely negative. Neither in the liver nor in the aorta could the author confirm changes equivalent to the ones described above. Thus, lecithin was shown to play no role in producing the changes noted with feeding egg yolk.

The possibility of all the lipids in question originating from the degeneration products of the liver cells themselves was excluded because no degeneration in the liver parenchyma appeared after 5 to 6 days of egg yolk feeding. The lipoid infiltration, however, was easily recognized.

On the basis of all the experiments discussed above, we concluded that there is probably only one substance that produced serious change in the liver when egg yolk is introduced into the rabbit. This substance is probably identical to cholesterin or its compounds. The changes in the aorta after egg yolk feeding, noted by Starokadomski and Stuckey, were also certainly caused by the cholesterin in the egg yolk. In badly injured aortic walls, Wesselkin observed considerable quantities of liquid spherical crystals that corresponded perfectly to the analogous elements in the liver parenchyma. This finding appears to us to be even more striking because it best confirms the identity of human atherosclerosis with the egg yolk-induced aorta changes described above. In the former, according to the investigations of Windaus, lipid materials regularly occur in copious quantities in the aortic walls.

Although all the experimental results cited above have caused us to believe that cholesterin or its derivatives causes the changes described in the aorta and liver, we still had no direct experiment that could confirm this. Kawamura has shown that the double refractive lipoids indeed arise from cholesterin introduced into the organism. However, Kawamura's experiments could not be clearly related to the experiments described above either in methodology or in quantity of associated spherical crystals. Hanes was the only author who described a similar picture in chicken embryos of copious infiltration of the liver with lipoid substances. As one of us has recently indicated (Chalatow\(^1\)), Hanes' observations appear to us to specify an analogy between the condition of the liver of chicken embryos nourishing themselves with egg yolk and the changes of this organ in rabbits artificially fed the same material.

Unfortunately, however, Hanes has not undertaken a more exact analysis of the lipoid materials deposited in the liver parenchyma. So we were not able to draw any conclusions from his work about artificial cholesterin infiltration and its significance in the origin of many pathological processes. We therefore recently initiated a new series of experiments in which rabbits were fed pure cholesterin over a long period of time. Although these experiments are not yet finished, we are presently able to draw a few conclusions which in our opinion could answer some of the questions raised by this work.

After only 4 to 8 weeks of feeding cholesterin (dissolved in oil\(^2\)) we produced in the experimental animals a rich infiltration of the entire liver parenchyma with the same liquid spherical crystals that one of us (Chalatow) has seen in the livers of rabbits fed egg yolk. These substances were also present in the aortic walls and in the spleen. The beginning of those

\(^1\) As indicated in the control experiments, the oil we used caused no essential changes in rabbit organs. In addition to cholesterin (0.5 to 0.8 pure cholesterin to each daily [sic: no units]) the rabbits also received their usual vegetable diet ad libitum.
pathological changes of the intima described by Stuckey and Starokadomsky could now be ascertained in the aortas of rabbits fed egg yolk. In one rabbit that received especially rich amounts of cholesterol, we even demonstrated free double refractive crystal structures in blood smear preparations. This fully agrees with Pribrams's chemical investigations of the blood, in which cholesterol is absorbed from the gut lumen into the blood and can be detected there in increased quantity after artificial feeding of cholesterol. In our experiments the adrenal glands were always very markedly enlarged, primarily because of an immense hypertrophy of the cortex, which contained a rich quantity of double refractive spherical crystals. An equally remarkable amount of these substances was also quite noticeable in the bone marrow.

On the whole, in all the experiments described above, the enormous infiltration of all organs with the semiliquid, double refractive crystal structures was quite surprising. On the basis of his well-known experiments, O. Lehmann has shown that these structures closely resemble the living state. This was also true in the generalized cholesterol steatosis produced in the original experiments.

The main result of the experiments just described was that we have now reconciled all the investigations of the authors named above. It became completely clear why only such foods as egg yolks or brain, for example, evoked pronounced and characteristic changes in the organism. Since these same processes are also observed on feeding pure cholesterol, there is no longer any doubt that it is specifically this substance that is deposited in the organism in the form of liquid spherical crystals and exercises an extraordinarily harmful effect on different organs.

At this point we shall not address the question of which chemical form of cholesterol is deposited in different organs in our experiments. Like Aschoff, if we assume that this substance occurs in the tissues only in the form of esters, then we assume that the pure cholesterol incorporated into the rabbit is in some way converted to cholesterol esters. Because, however, the precise chemical events in the absorption of cholesterol are not fully known, we must leave the answer to future physiological-chemical investigations.

At present, the only important fact seems to be that pure cholesterol and cholesterol-containing foods cause the appearance of a predictable pathological process in rabbits. To answer the question about whether other types of animals are equally sensitive to this substance, we have started feeding experiments with white rats, and one of us has further investigated a few guinea pigs in this regard. Rats and guinea pigs were fed large amounts of egg yolk (blended in cow’s milk). The results of this feeding were quite different in the two types of animals. One could ascertain in the guinea pigs the same processes observed in rabbits fed with egg yolk, specifically, an enormous cholesterol infiltration of the inter nal organs. These same processes were completely absent in the rats. Neither the liver nor the aortic walls in the rats showed any changes. Further, absolutely no traces of deposition of liquid crystals, so characteristic of rabbits, could be detected in the liver. Equally negative were the rat experiments in which the experimental animals received a combination of alcohol and egg yolk as nourishment. Only after feeding the rats a very rich amount of brain substance did we see an accumulation of small, crystalline lipoid substances in the liver parenchyma, even though they were present in a limited quantity.

On the basis of the rat experiments described above, we conclude that the harmful effect of cholesterol-rich nourishment is not expressed equally in all types of animals. The underlying cause of such a striking difference in the behavior of two kinds of animals that are rather close in the zoological hierarchy still remains completely unclear. It is possible, however, that we shall produce in future experiments a harmful effect of cholesterol on rat organs, perhaps if this substance is introduced in combination with substances that are likely to have other poisonous effects.

The fact that cholesterol has different effects on different animals, even closely related ones, raises the question to what degree the results described above for rabbits are valid for human pathology. As is noted in the literature, the human body does not seem to be indifferent to cholesterol. In many pathological changes of both localized and generalized nature, one finds cholesterol and its compounds deposited in the tissues in great quantity. Many authors have even associated fluctuations in blood cholesterol content with the origin of some pathological processes.

As we know, however, cholesterol is especially plentiful in the bile and is the dominant component of all gallstones. We have experimentally ascertained an enormous quantity of cholesterol crystals in the bile of a rabbit that was fed copious amounts of cholesterol. In contrast to the reports of Jankau, our finding proves that cholesterol introduced into the body is partially secreted with the bile. Our finding also confirms the views of Chauffard, who associated gallstone illness with an increased cholesterol content in the body.

Particularly Chauffard is known to have described explicit observations about the importance of cholesterol in the origin of many forms of illness.

In addition to gallstone illness, Chauffard includes atherosclerosis among those illnesses in which cholesterol and its derivatives are especially important. With regard to atherosclerosis, the results of the experiments described in this paper appear to agree fully with Chauffard’s clinical observations.

Certainly many of the questions now raised about cholesterol metabolism and its pathology require further experimentation. To answer these questions different methods of investigation will be used, particularly in the area of physiological chemistry and
pathological histology. This article only has the purposes of: 1) repeating in brief our results and those of several other Russian researchers, and 2) correlating the results with each other and with the results of those authors who have recently established many new facts in the science of lipoid substances. Given the current, wide-ranging interest in this field, and because these substances have been shown to elicit the expression of pathological processes that are perhaps analogous to human pathology, it seems timely to initiate exhaustive studies in experimental pathology.

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