Observations CONCERNING THE RELATIONSHIP BETWEEN CHOLESTEROL METABOLISM AND VASCULAR DISEASE *

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It has long been known that the intima of the vessels is particularly disposed to fatty change: Virchow took a particular interest in this subject, and in his treatise on scientific medicine he dealt with it at length. He differentiated the simple fatty changes in the cells of the intima, and the roughness which results therefrom, from the atheromatous foci which develop in the depth of the same intima.

We believe to-day, however, that the two processes, which even in Virchow's time had been classed together as atherosclerosis of the intima (*Zellulärpathologie*, 4 Auflage, 1871, p. 462), are, in fact, one and the same.

In view of the evidence brought forward by Adami, and concurrently by myself (*Proceedings of the Royal Society*, B, lxxviii), it would appear that the doubly refractive fat droplets in the intima should be regarded as cholesterol esters and cholesterol oleates—it is thus appropriate to speak of a fatty degeneration of the vessels as due to cholesterol esters. This view received further support from Panzer's chemical investigations, which were published in the same year as our own papers. The views of Windaus, who carried out investigations somewhat later (dealing particularly with atherosclerosis) also tended to support our opinions. However, the conception of a primary fatty change as the first departure from the normal in atheromatous disease of the vessels has not been universally accepted. I would like to point out here that we have never stated, as so many persons have alleged, that the fatty change in the intima is a *pure* cholesteatosis—it would only be possible to think this if one had hurriedly read the reports of the International Congress in Budapest (*Ziegl. Beitr.*, 1910, xlvii, 43) or Kawamura's monograph (p. 110). On the other hand, we may state that among the visible fatty deposits the cholesterol esters appear to predominate.

ATHEROMA AS A "CHOLESTEROL STEATOSIS"

From the histological standpoint it is only possible to speak of a fatty change when fat is *visible*. The term "cholesterol steatosis" must necessarily remain a morphological conception carrying no chemical or quantitative implication. The visible fat in the intima must, however, in view of its reactions, be regarded as a cholesterol ester. It is, of course, understandable that chemical analysis, which also accounts for the *invisible* fat, will give different values, but we must remember that this is able to give us 55 to 60 per cent. cholesterol esters and 23 to 26 per cent. of free cholesterol in cases of well-marked atherosclerosis (Schoenheimer). Thus, even from a chemical standpoint, it is reasonable to speak of a cholesterol steatosis, since the use of this term emphasizes the difference from the fatty compounds of glycerol, which only appear in small quantity in the total fat. As far as is known, neutral fats play no part in the fatty changes in the intima.

In addition to cholesterol esters and cholesterol, however, both phosphatides and cerebrosides have been found, and Kutschera-Aichbergen has attributed a special role to the former. It may be seen from Schoenheimer's curves that this is really not the case. Furthermore, one may

point out that the cerebrosides have likewise no special function, since these, together with cholesterol esters and phosphatides, may all be found in the normal aorta. In the so-called atheromatous process there occurs, to begin with, an increase of all three kinds of lipoids; this is clearly demonstrated by the curves of Schoenheimer and the figures given by Kimmelstiel. It may be observed, nevertheless, that the increase is most marked in the cholesterol esters (including the free cholesterol). In severe cases of atheroma the cholesterol (free and combined) may increase 112.8 times, the phosphatides 39.5 times, and the cerebrosides 38.5 times. One is therefore justified in describing the pathological state of atheromatosis as a cholesterol steatosis, especially since the phosphatides and cerebrosides in the fat mixture are not readily demonstrated by any physical or staining methods. A chemical pathologist will always keep the presence of these substances in mind, but their part in the disease process is relatively unimportant.

MECHANISM OF LIPOID DEPOSITION

If we are to-day better informed as to the most important chemical substances occurring in the fatty degenerations of atheroma, we yet remain in some ignorance as regards the cause and nature of this condition. In attempting to throw light on these matters I shall find it necessary to discuss the origin of the lipoids. In this matter the methods of histology will prove helpful, for they show that the deposition of lipoids occurs first of all in the intima. The systematic researches of Nora Nordmeyer have shown that the media is only involved in the deposition of lipoids in those parts where the deeper elastic layers of the intima—those lying next to the media—are particularly loosely knit. This is found to be the case in the aorta, the carotids, and the coronary arteries. In other parts the deposits of fat are confined to the intima. What is the explanation of this lipoid infiltration of the intima? The explanation must surely be sought in the blood.

We have known since Virchow's time that the intima and the inner two-thirds of the media of the large vessels derive their nourishment from the blood stream. A continuous stream of lymph evidently passes from the circulating blood into the vessel walls. The experiments of Lange support this conception, which suggests that the lipoids are brought into the vessel walls from within the lumen. In these circumstances the deposits will occur first in those places where the physical conditions are most favourable. If we observe the places in which they occur in man, we shall notice that they are usually in the so-called elastic limiting layers; in the aorta, for example, in the intermediate elastic layer, in the coronaries in the elastica interna (internal elastic lamina)—in every case they occur at the border of intima and media. Now this border tissue is formed of a close meshwork of elastic fibres or of a more homogeneous layer. Unfortunately we have no information concerning the intimate structure of this elastic border tissue, but we may reasonably assume that some cementing substance is present to hold the elastic fibres together. Similarly it may be shown that the elastica interna is also a mixture of tissues and not homogeneous. It is formed of a series of layers which exhibit differences in their staining reactions. I myself am of the opinion that the cementing substance which lies between the elastic fibres, and by which the elastic tissue is thickened into a definite elastic layer, or lamella, is in fact the site of the lipoid deposits. Although it might appear at first sight that the *whole* of the border tissue had stained with Sudan red, closer examination reveals that the fatty changes of the intima always begin in the depths of this layer, close to the border against the media. It is reasonable that this

* Contribution to a discussion in the Section of Pathology at the Centenary Meeting of the British Medical Association, London, 1932.

variety of fatty change should have been termed "interstitial" in contrast with the cellular variety.

If lipid substances are derived from the blood and reach the intima by way of the lymph, to be precipitated in the fine meshwork of the intermediate elastic layer, then it would be reasonable to expect that the route by which this absorption occurs would become progressively more obvious as the deposits increased; in other words, the lipoids would approach nearer and nearer to the lining of the vessel. This is in fact the case, and is readily understood, since the fine network of the elastic layers will be increasingly congested by the accumulating lipoids, so that the rate of absorption gradually decreases. The greater the accumulation of lipoids in the interstitial tissue the more obvious does the presence of drop-like deposits in the cells of the intima become. The coincidence of these two phenomena is so frequent that it has suggested to some people that the whole process is cellular in origin. It is thus easy to understand why the condition was at one time looked upon as a failure of compensation, in which the cells were no longer able to resist a fatty change; we know to-day that the process is merely an infiltration. Furthermore, it would be quite reasonable to suggest that the cellular fatty changes only occur when a certain degree of infiltration of the interstitial substance has taken place.

Many observations, including those of an experimental nature, tend to support this view. All tissues which have undergone fatty change—both cellular and interstitial—exhibit a more or less marked turgescence, which is evidence of imbibition. In the interstitial tissue this may be detected from the increased separation of the elastic fibres. In a cross-section the elastic layer then appears to be in a state of granular degeneration. If at the same time the media is also diseased, the cementing substance between the collagen and elastic fibres also exhibits a lipid infiltration and fatty change. In association with this there may at times be seen a fatty degeneration of the muscle fibres. As has already been stated, these lipid infiltrations of the media, which only occur in special sections of the vascular system, play no part comparable to the fatty changes of the intima.

THE INFLUENCE OF AGE

If, then, we are able to look upon atheroma as an imbibition process from the blood plasma occurring in the intima—a conception for which histological evidence is forthcoming—we shall find it necessary to inquire why such imbibition produces such varying results at different periods of life. There is no doubt that this imbibition begins in early childhood, but it does not lead to pronounced atherosclerosis—that is, *endaortitis deformans*—until a considerable age has been reached. Special circumstances must therefore be present in each period of life to bring about that form of the disease which is peculiar to it. We are able to distinguish to-day between the types which occur in babyhood, at puberty, and in adult life. It has been shown that in babies the large mitral valve and the root of the aorta are most often affected, while in the second and third decades the thoracic, and in later life the abdominal, aorta are most often involved. We might say, in fact, that the atheromatous changes spread from the aortic root into the abdominal aorta, and later into the vessels of the limbs. Similar spreading lesions may be observed in individual vessels—as, for example, in the coronary arteries of the heart. In the latter the main branches, generally on the left side, are first affected, while the medium-sized and smaller branches, particularly the points of division, become diseased later. There is no doubt whatever that this method of spread is a phenomenon associated with growth and of the increasingly important functions of the

peripheral vessels with the advance of years. It is not only a question of a spread of atheroma to the periphery occurring in stages corresponding to the various stages of life. We have, in fact, to deal with alterations in the disease picture, both microscopical and macroscopic. Whereas in babyhood and at puberty we find a simple lipid imbibition in a slightly thickened intima, which coincides with the growing period of the vessels in age, when the vessels are undergoing regressive changes we find the nodular and irregular, or "geographical," thickenings of the intima which constitute a process recognized by the name of *endaortitis* or *endarteritis deformans*.

These lipid deposits are generally much more marked than those which occur in youth, but the thickening is due not only to these lipid accumulations, but also to associated swelling and proliferation in the adjacent intima. These are to some extent caused by the invasion of protein substances also derived from the blood stream (hyaline swelling or hyalinosis). In association with this infiltration a true hypertrophy and new formation of collagen and elastic fibres occurs. These lesions are of a porcelain-white colour on the surface, but when incised will be seen to exhibit more or less marked lipid infiltration in the deeper structures. There is experimental evidence to show that every lipid infiltration of the intima, when it has been present a sufficient time, causes a reactive hypertrophy of the adjacent intimal tissues.

PATHOLOGY OF NECROTIC CHANGES

Why do the changes described above occur in that period of life which constitutes decline? We must assume that as age advances the overstretching of the vessels and the associated changes in the intima favour swelling of fibrous tissues and the proliferation of cells. In any case the general molecular disintegration which the intima suffers during life conduces to severe disease of this structure, and this may be contrasted with the lipid infiltration of the intima of the aorta which occurs in younger people. The whole process of imbibition is one in which lipid substances become deposited. In young persons it remains at a certain level, or may even decrease, but in older persons it advances irresistibly because of the physico-chemical changes which have occurred in the walls of the vessels; yet old age alone does not bring about any atherosclerosis of the type of an *endaortitis deformans*, though an increasing absorption of lipoids is a factor in its production.

The chemical investigations of Schoenheimer, who examined the lipid content of the intima, support this view. He was able to show that the percentage of free cholesterol in the intimal fat remained practically the same although the arteriosclerotic process was getting worse. The percentage content of the other lipoids, however, particularly of the cholesterol esters, rapidly increased. This may be explained by the increase in the absorption of cholesterol esters from the blood.

The larger the atheromatous patches with their contained lipoids become, the more easily does necrosis of tissue occur in them. One might say, in fact, that the tissue is choked with lipoids. As soon as necrosis begins, though, visible changes occur in the lipid also. The cholesterol in combination is liberated, and forms the well-known acicular crystals shining brightly in the debris of the fatty substances. At the same time the usual sclerosis of the atheromatous foci from a deposition of calcium salts begins—the process to which the term "atherosclerosis" has been applied. We do not understand the exact nature of this calcification. Histological preparations show an increasing diffuse or granular calcium staining at the edges of the necrotic areas. At one time I shared the opinions of Klotz, believing, in view of

the investigations of Kawamura, that calcium soaps were formed, presumably as combinations with the fatty acids which had been liberated by the break-up of the cholesterol esters. The very careful experiments of Schoenheimer failed, however, to supply positive evidence in support of this hypothesis, so that it must be considered possible that the calcifications are independent of the lipid deposits and perhaps merely a consequence of necrosis. This type of calcification is termed "secondary calcification," or more often "calcification of the intima."

We know that in addition to the lipid substances and the protein (hyaline), which has been mentioned, certain other constituents of the blood plasma may penetrate the vessel walls. First among these must be mentioned the slimy or mucinous substance which is seen in considerable quantity, even in normal circumstances, in the walls. It is difficult to decide whether we have to deal with an increase of the amount normally present or with a swelling of that which is already there. It is at least certain that this mucinous change is most marked in the media. Evidently the fluid carrying the mucin, or that which causes the mucinous interstitial substance already present to swell up, is able to traverse the elastic lamina between intima and media more easily than can the lipoids. It is only in those places where the intima has already become thickened from other causes that this substance is precipitated. The mucinous changes of the media are thus in every way comparable with the atheromatous changes of the intima. Furthermore, the distribution of these changes throughout the vascular system is identical.

CALCIFICATION OF THE MEDIA

We must now discuss another matter to complete the picture of atheroma. This is the calcification of the media, the so-called primary calcification of the vessel walls. It is not preceded by any lipid change, such as happens in the intima, but is a peculiar calcifying process in the muscle fibres, and the collagen fibres around them, which finally leads to necrosis. The elastic fibres usually continue to stain quite well, but are seen to be stretched out, so that calcification of the media evidently occurs while the vessel is under tension. The foci of calcification are usually to be found immediately adjacent to the internal elastic lamina or the limiting elastic tissue. This is at times undergoing a fatty change on the side nearer to the lumen of the vessel, while towards the media it is often partly calcified.

It is thus evident that the calcification which occurs so easily in the media immediately adjacent to the elastic layer is due to the peculiar physico-chemical properties of the former tissue. There is no doubt that the advance of age is able here, as elsewhere, to favour this change. It is rather remarkable, however, that these processes do not tend to occur in the aorta, or the vessels of the brain and heart, but rather in the limbs, especially the tibial vessels. The special neuro-muscular conditions here must undoubtedly be a factor in the development of calcification. It is not yet possible to decide whether substances which are formed in the limbs as a result of activity may be able to penetrate the media of the limb vessels with particular ease and exercise a local influence therein. Although such substances may exist we know nothing of their chemical constitution. It is not unlikely that, with the deposit of the cholesterol, active ergosterin also comes into the walls of the vessel and consequently irradiations of the body encourage the calcification of the media (Windaus). At any rate, fatty degeneration in the intima and calcification of the media have a much closer connexion than has been thought during the last few decades. There is no ground for differentiating between usual arteriosclerosis and Mönckeberg's vessel disease.

GENERAL CAUSATIVE FACTORS

X-ray photographs of the arterial system in a dead body, taken by Rühl, also support this contention. From such evidence one may be certain of the close connexion between the various forms of degeneration of the vessel walls: it is likely that this would be the case since a common aetiological factor (a more or less marked absorption from the blood lymph) is present in all. Rühl's figures show that the vessels in various parts of the body exhibit considerable variations in the extent of the morbid changes which have occurred in them. In one case a vessel will have suffered marked fatty changes of the intima, while in another the same vessel will exhibit considerable calcification of the media.

As regards the aetiological factors in the development of atheroma the changes described as occurring in the vessel walls cannot take place unless the substances which produce them are present in sufficient quantity in the blood plasma. We know quite well that the amount of cholesterol in the blood plasma varies within wide limits, and we also know that it is essential to differentiate between the free and the combined substance. One might therefore be inclined to ask whether any relation can be established between the atheromatous changes in the intima and the cholesterol content of the blood and lymph as a result of the examination of the dead body. Let us here consider a few cases in point. It has been customary to suggest that the atheroma of babies is due to the high cholesterol content of their food—this problem is still under investigation. With regard to the atheromatous changes of puberty, the part, if any, which is played by the ductless glands is unknown, though the increase of lipoids in the suprarenals and in the interstitial cells of the testis occurring at puberty would suggest that some such mechanism is present. One must be satisfied with the inference. In adults we have to blame either the primary disease or the diet for the increase of atheromatous changes. All diseases which are associated with a considerable increase of the blood cholesterol, such as diabetes, cardiac disease, and hypertonia, lead to an increase in the deposit of lipid. The full picture of end-aortitis deformans will not, however, develop until the persons concerned have reached their period of decline. We do not know with certainty whence the blood cholesterol in these diseases is derived. It appears most likely that it originates in the food. This hypothesis is also supported by feeding experiments, with cholesterol in oil, which have caused a more or less marked atheromatous change in the heart valves and the intima of the great vessels, these being particularly well developed in rabbits. But we must not forget that the excretion of cholesterol through the liver and the bile occurs differently in the herbivora and the carnivora. For this reason one should not attempt to apply the conclusions of experiments with rabbits too freely, and their interpretation in human beings must be accepted with reserve. It is conceivable that the endogenous formation of cholesterol which has been shown to occur in the carnivores may become disturbed. In this way an over-production might be possible, so that a condition which could be described as a cholesterol gout (Schoenheimer) might arise. If this were the case it would be reasonable to look upon atheroma as a metabolic disease and not as a disorder of nutrition.

Mechanical Factors

Purely chemical factors, however, are not alone the cause of atheromatous disease. The facility with which the blood serum penetrates the vessel walls is a most important consideration, and this depends, first, on the blood pressure, and, secondly, on the structure of the vessels. With regard to the former, the causal relation

between blood pressure and atheroma is supported by convincing evidence. The most striking is perhaps the presence of atheroma in the ascending aorta in cases of aortic stenosis. Almost equally convincing is the development of lesions in the pulmonary artery in the presence of left-sided valvular disease.

The localization of the lesions is not determined by the blood pressure alone, but is also influenced by the effects of the blood stream in particular places. All so-called weak spots, such as the scarred remnants of the ductus arteriosus, and all places in which the current of the blood most easily causes a loosening of the structure of the intima, are liable to be affected at an earlier date and more severely than are other parts. We must include in these the points at which branch vessels are given off, and those, such as the surface of the mitral valves or of the sinus of Valsalva, on which the blood impinges with great force, together with those parts which are most firmly fixed and therefore exposed to a correspondingly severe shock. The mechanical factor is thus seen to be of great importance in determining the localization of the lipid infiltration. It is not yet possible to say to what extent, if any, variations in the tension of the vessels concerned—controlled by the nervous system—affect the morbid changes.

In reviewing the whole picture of the development of atheroma one is forced to the conclusion that the precipitation of lipids is the most important factor. As a corollary to this one has to admit that all inquiries into the nature of this condition must, of necessity, be intimately concerned with the problems of cholesterol metabolism.

THE BIOCHEMICAL SYNDROME OF URAEMIA

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In recent years, following certain Continental workers (Volhard,¹ Widal and Javal,² Ascoli,³ Strauss,⁴ and others), most clinicians have modified their views on the nature of certain so-called "uraemic" manifestations in man. It must, however, be admitted that our present conception of the uraemic syndrome is a very confused and unsatisfactory one, as is evidenced by the continued use of such terms as "acute uraemia," "chronic uraemia," "true uraemia," "false uraemia," and so on. The object of this paper is to distinguish the true biochemical syndrome of uraemia from certain allied conditions.

The complete removal of both kidneys from the young and healthy animal constitutes the only satisfactory method of inducing pure and uncomplicated renal failure. It has long been known, since the studies of Prévost and Dumas,⁵ that such a procedure is followed by the progressive accumulation in the blood of those metabolic end-products which would normally be excreted in the urine. Such accumulation is followed by a profound upset in the blood and tissue chemistry, which in turn produces a series of clinical phenomena eventually terminating in death. Strictly speaking it is to this series of phenomena, and to it alone, that the term "uraemia" should be applied. When we come to consider the question of uraemia in man, however, certain important difficulties arise which cannot on any account be overlooked. Many diseases are accompanied by a functional disturbance of the kidney, which tends, if severe, to end in a condition of uraemia apparently similar to that following total nephrectomy in animals. These diseases

(for example, chronic glomerulo-nephritis, malignant renal sclerosis, gross bilateral disease of the kidney, and acute nephritis) are, however, invariably accompanied by certain important *extrarenal* factors, which produce a complex clinical picture of their own. The most important of these extrarenal factors are: vascular hypertension; diffuse arteriolar and arterial disease, with resulting structural changes in organs other than the kidney; myocardial degeneration; and certain obscure vasomotor phenomena not yet fully understood. For this reason it is practically impossible to encounter in man a case of pure uraemia free from all disturbing factors, and comparable to that following total nephrectomy in animals. Such a case would be of extreme interest, as it might be expected to aid in deciding what features of the familiar clinical picture are truly uraemic and what are the associated alterations in blood chemistry.

A case will here be described of a young and healthy man who lived for ten days after total removal of all his kidney substance, and who, during this time, was studied from the clinical and biochemical aspects. He thus resembled in all respects the laboratory animal in whom uncomplicated uraemia is produced by total nephrectomy. A study of the literature has failed to reveal any parallel case, though patients with anuria who have lived for long periods are not uncommon. Thus Myers,⁶ in reporting a case of apparently complete urinary obstruction of thirty days' duration, made an extensive survey of the literature, and could only collect nineteen cases of complete anuria with a duration of twenty to twenty-nine days. A few cases, however, are reported of anuria of even longer duration (up to sixty days, Baily⁷). Most of the cases are due to renal calculus or "toxic nephritis," occasionally to ureteric obstruction or "reflex anuria." All the cases are reported as curiosities from the clinical point of view, and in none was any real attempt made at biochemical investigation. Such cases are of course complicated by the condition causing the anuria. Many workers have attempted to investigate prolonged anuria due to mercurial nephrosis, but in such cases the profound general effect of the poison makes accurate study of the renal condition impossible.

A CASE OF PURE URAEMIA

A healthy young man, aged 22½, was kicked in the left loin when playing football. Thereafter he suffered severe pain, and some days later was brought to hospital with heavy renal haemorrhage and severe colicky pains from the passage of blood clot. Conservative methods were tried for some days, but failed to arrest the haemorrhage. The kidney was explored and found to be hopelessly ruptured, the rupture involving the hilum and necessitating removal. The patient lived for ten days after operation without secreting a drop of urine, and then died of uraemia. Cystoscopy, confirmed by post-mortem examination, showed that he was the subject of congenital absence of the right kidney and ureter. The bladder was triangular in shape, as if the left side only had developed. There was no sign of any remnant of kidney or ureter on the right side. The temperature rose to 101° F. after the operation, but gradually settled down, and for the last four days remained steadily subnormal (97° F.). The pulse rate rose to 140 after operation, but settled down and remained steadily between 90 and 110 until death. The blood pressure did not rise beyond the normal limits. It was 116/62 on admission and 110/40 on the day before death. There was thus no hypertension, but a marked fall in the diastolic pressure.

There was no apparent alteration in the size of the heart, and the rhythm was normal throughout.

Symptoms

For four days following the operation the patient remained in perfect health and was quite happy. He received nothing by mouth except water and calcium chloride. Forty ounces of 5 per cent. glucose in saline were given by rectum each day. On the fifth day the first definitely uraemic symptoms