ARTERIOLAR INVOLVEMENT IN DIABETIC RETINOPATHY*

BY

NORMAN ASHTON

Department of Pathology, Institute of Ophthalmology, London

IN recent studies of diabetic retinopathy much emphasis has been placed upon the pathological changes on the venous side of the retinal circulation, and little attention has been directed towards the arterial involvement, which is now generally regarded as a late, super-added, non-specific development, attributable to arteriosclerotic or hypertensive mechanisms. In long-standing diabetes it is well recognized that arterioles throughout the body are particularly prone to undergo hyaline degeneration and occlusion; such changes are strikingly apparent, for example, in the small arteries of the pancreas and in the glomerular arterioles of the kidney. This arteriolar degeneration may be remarkably severe; indeed it has been stated that a substantial deposit of hyaline material in the arteriolar walls is characteristic of diabetes and is strong presumptive evidence of its existence when found histologically (Bell, 1946). Renal arteriosclerosis, for instance, is over three times as frequent in normotensive diabetics as in normotensive non-diabetics and the lesions are much more severe in the former (Bell, 1952). Our own experience in the histological study of extra-ocular tissues, removed *post mortem* from diabetic subjects, is completely in accord with this view.

In an extensive investigation of the problem of diabetic retinopathy now being carried out at the Institute of Ophthalmology, a study has been made of the changes on the arterial side of the circulation. It has been found that in the later stages of diabetes, the retinal arterioles may be affected as severely as the extra-ocular arterioles, and that the arteriolar hyalinization and obliteration is responsible for a characteristic type of new channel formation within the retina and subsequently for the final and total destruction of the retinal capillary bed.

It is probable, therefore, that this severe degree of arteriolar involvement should not be regarded merely as a super-added hypertensive complication, but as a characteristic, late development of the diabetic process itself. It is the purpose of this paper to report these findings, which have so far been only incompletely described (Ashton, 1951), and, as would now appear from the examination of more extensive material, in part erroneously interpreted.

Pathological Findings

The foregoing observations were made on injected and stained retinæ removed *post mortem* from diabetic subjects; the techniques employed have been described in detail elsewhere (Ashton, 1949, 1950).

It was noted early in the investigation that, in cases of established diabetic

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retinopathy, small or large areas in the retinal capillary network failed to fill with the injection fluid. It was realized that, while these defects might well have been due to occlusion of the lumina of diseased vessels, artefacts due to failure of injection could not be excluded as a cause. As more specimens were examined, however, it was found that this feature was not apparent in either normal retinae or in the early stages of diabetic retinopathy. Furthermore the avascular areas were constantly found to lie in the neighbourhood of the retinal arteries (Figs 1 and 2), except in the most severe cases where large areas of the capillary network were often obliterated.

FIG. 1.—Diabetic retinopathy. Artery on the left, vein on the right. Microaneurysms are seen on the venous side of the capillary circulation. An early stage of arteriolar occlusion is present and a few terminal arterioles and precapillaries are completely obstructed. There is a consequent patchy loss of the capillary bed around the artery. Injected Indian ink. PAS stain $\times 44$.

FIG. 2.—Diabetic retinopathy showing a more advanced stage than that seen in Fig. 1. Artery on the left, vein on the right. Haemorrhages and microaneurysms are present; in the centre of the picture they are seen on an arteriole. The arteriolar side of the circulation is more extensively atrophied than in Fig. 1. Injected Indian ink. PAS stain $\times 44$.

It was also noted that these peri-arterial zones of capillary atrophy were associated with two other changes, namely :

(1) Narrowing or complete occlusion of the terminal arteriolar branches and precapillary arterioles.

(2) New channel formation on the venous side of the capillary network.

(1) **ARTERIOLAR CHANGES.**—In techniques involving examination of the flat retina removed *post mortem*, there are many ways in which artefacts may arise, and, as has already been emphasized (Ashton, 1952), one must be particularly careful in interpreting narrowing of vascular junctions as evidence of pathological change. In order to obviate this difficulty, retinæ mounted flat after injection with Indian ink were stained with the periodic acid-Schiff method, which, as Friedenwald (1948) was the first to show, stains the vessel walls an intense red, thus making it possible to determine whether narrowing of the lumen of a vessel is associated with changes in the adjacent vessel wall. Using this technique, it was found that, in retinæ in which diabetic retinopathy was fully established, the lumina of the terminal arteriolar branches and the precapillary arterioles were often markedly

FIG. 3.—Terminal arteriole showing hyaline thickening and narrowing of lumen at point of origin. Injected Indian ink. PAS stain $\times 508$.

narrowed, particularly, but not exclusively, at their points of origin and in the early part of their course (Fig. 3). These changes were frequently associated with an irregular thickening of the vessel walls, which sometimes imparted a corkscrew appearance to the ink in the lumen (Figs 4 and 5, opposite).

A comparison of retinæ showing different degrees of retinopathy indicates that this pathological development progresses to complete obstruction of the vessel, so that gradually fewer and fewer terminal arterioles and precapillary branches remain patent ; ultimately the arterial side of the capillary network closes down and

the capillary bed, starved of blood, atrophies and disappears. The remnants of these degenerative vessels stain but faintly with the PAS method and can be followed only imperfectly in their course, appearances which rule out the possibility that normal vessels have failed to fill on injection. In the final stages of obliteration PAS staining reveals little trace of them.

(2) **NEW CHANNEL FORMATION.**—In areas where the arterial side of the capillary network was undergoing obliteration, numerous dilated loops could be seen,

FIGS 4 and 5.—Terminal arterioles showing hyaline thickening and corkscrew coiling in early part of vessels' course. Injected Indian ink. PAS stain $\times 172$.

situated roughly midway between the artery and vein, projecting towards the artery and communicating with the venous capillary circulation (Figs 6 and 7, overleaf).

PAS staining demonstrated that the loops were connected *via* obliterated vessels with the capillaries of the arterial side (Fig. 8, overleaf).

The dilated loops were, in fact, new channels which had developed on the venous side after occlusion of a proportion of the terminal and precapillary arterioles. They were frequently compressed into grotesque Y-shaped and S-shaped forms, possibly as a result of destruction of the supporting framework of the retina and of the distorting effect of oedema and exudates. As has already been indicated, when the arterial supply to a particular area becomes obliterated then the capillary bed, together with these new channels, undergoes total atrophy (Fig. 9, overleaf).

In a recent paper these loops were erroneously described as examples of intra-retinal new vessel formation (Ashton, 1951), but it is now clear that the dilated channels are not of this nature; indeed, intra-retinal new vessel formation, although it undoubtedly occurs, is a much less common development.

The formation of new venous channels in the diabetic retina has also been described by Michaelson and Campbell (1940) and by Ballantyne (1945), but these authors believed that the new pathways resulted from venous congestion or venous block. Such by-passes may possibly develop from venous occlusion, but the majority appear, in our experience, to follow obstruction on the arterial side.

Discussion

The severe degree of arteriolar disease, the associated development of new channels, and the final obliteration of the capillary bed, combine to present a pathological picture which has not so far been paralleled in numerous examinations of injected retinæ from non-diabetic cases with hypertension

FIG. 6.—Diabetic retinopathy showing an artery in the centre of the picture. The arterial side of the circulation is undergoing obliteration and numerous dilated new channels may be seen, situated roughly midway between the artery and vein, projecting towards the artery, and communicating with the venous side of the capillary circulation. Note the characteristic S-shaped and Y-shaped forms. Injected Indian ink. PAS stain $\times 44$.

FIG. 7.—Diabetic retinopathy. Artery on the left, vein on the right. The capillary bed around the artery is atrophic, because of obliteration of the majority of the terminal arteriolar branches. New dilated channels may be seen in the centre of the capillary bed. Near the centre a thick-walled micro-aneurysm is present. Injected Indian ink. PAS stain $\times 44$.

or arteriosclerosis. There is no apparent reason, however, why a prolonged and severe degree of arteriolar obstruction, from sclerosis or spasm, should not give rise to similar sequelae in non-diabetic subjects. These points await elucidation in the examination of further material, and at present one can only say that they must be comparatively rare. This is probably because arteriosclerotic changes are usually less severe in the non-diabetic than in the diabetic, and that occlusion through spasm is too sudden to allow the formation of new channels and too transient to lead to permanent obliteration

FIG. 8.—A terminal arteriole crosses the centre of the field, and new channels forming grotesque loops may be seen in the lower half. They are connected with the arteriole *via* narrowed or obliterated precapillary arteriolar branches. Injected Indian ink. PAS stain $\times 120$.

FIG. 9.—Diabetic retinopathy. Artery on the left, vein on the right. Complete occlusion of the arteriolar branches has led to total atrophy of the capillary bed, including any new channels which may have existed. Injected Indian ink. PAS stain $\times 44$.

of the terminal arterioles and precapillary vessels. On the other hand, it is clear that a severe degree of arteriolar obliteration occurs in diabetic retinopathy with sufficient frequency to be regarded as a characteristic feature of the disease in its later stages.

Taking these new findings into consideration, the sequence of structural

changes in diabetic retinopathy would now appear to be as follows :

- (1) Venous engorgement (ophthalmoscopic evidence).
- (2) Distortion of capillary network with localized capillary looping. Micro-aneurysm formation occurring frequently at the sites of the capillary loops (Figs 10 and 11).
- (3) Formation of haemorrhages and exudates.
- (4) Hyaline constriction of terminal and precapillary arterioles (Figs 3, 4, 5, and 8).
- (5) Partial atrophy of capillary bed on the arterial side (Figs 1, 2, 6, and 7).
- (6) Formation of new channels in capillary bed on the venous side (Figs 6 and 7).
- (7) Complete obliteration of arterioles and disappearance of capillary bed (Fig. 9)

FIGS 10 AND 11.—Two stages in the development of micro-aneurysms from capillary loops. Fig. 10 shows a varicose loop with a cap of dense PAS-positive exudate ($\times 406$). In Fig. 11 the opposing walls of the loops have fused and the thickened laminated exudate surrounds the aneurysm ($\times 360$).

Both forms of true new vessel formation—that arising in association with retinitis proliferans after vitreous haemorrhage, and that arising as rete mirabile in ischaemic areas—may occur either early or late in the disease. The complications of glaucoma, retinal detachment, and atrophía bulbi are not of importance in the present discussion.

It will be seen from the above order of events that the arteriolar involvement plays no small part in the unfolding of the final disastrous picture of diabetic retinopathy, for it would appear to be almost entirely responsible for the complete obliteration of the capillary blood supply to the retina.

It is to be remembered that the arteriolar changes described above were observed in the late stages of diabetic retinopathy, and that any inferences attempting to relate them to the early stages of the disease must necessarily be purely speculative. Bearing this limitation in mind, it would nevertheless appear that these pathological changes can be interpreted in only two ways : namely, that the obliteration of the arterial side of the capillary circulation is caused by an occlusive sclerosis developing as a late manifestation of the retinopathy, and is thus unrelated to the early stages of the disease process, or that the changes represent a structural alteration in vessels previously involved in chronic functional disorder.

The weight of clinical and pathological evidence supports the view that the arteriolar changes merely represent a late stage of development in the retinal disease, for the ophthalmoscopic picture of arteriospastic and arteriosclerotic retinopathy differs considerably from that of diabetes, and, as has already been noted, arteries of comparable calibre elsewhere in the body are particularly prone to undergo hyaline degeneration in diabetes of long duration. Furthermore, no hyalinization or other structural change can be found in the retinal arterioles when the early stages of diabetic retinopathy (micro-aneurysm formation, haemorrhage, and exudates) are already established. It is now known, therefore, that the retinopathy is not initiated by precapillary sclerosis as suggested by Cristini and Tolomelli (1946); indeed, the apparent normality of the arterial side of the circulation and the involvement of the venous side have frequently been contrasted with the opposite findings in hypertensive disease. It was upon this distinction that Ballantyne (1945) based his differentiation of the two retinopathies, the pathological changes in the diabetic type being primarily in the capillaries and venules, while those in the hypertensive type were primarily in the arterioles and precapillaries. Michaelson (1948) carried the argument a stage further by stating that the difference between these pathological patterns was related to the developmental distinction between arteries and arterioles on the one side and the vein-capillary unit on the other.

It has recently been suggested by Duguid and Anderson (1952) that hyaline arteriosclerosis does not represent a degeneration of the vessel wall, as is commonly supposed, but is the product of a deposit of haematogenous origin in the lumen of the vessel. It is possible, therefore, that hyalinization of the arterioles in diabetes may be simply a manifestation of the disturbance of mucopolysaccharide metabolism which is known to occur in this disease (Friedenwald, 1948, 1950; Jacobs, 1949; Ashton, 1949, 1951; Rifkin and Petermann, 1952). Indeed, Warren and LeCompte (1952) see in this abnormality an explanation of all the important lesions characteristic of diabetes.

It may be, however, that in the study of diabetic retinopathy attention has been too narrowly confined to changes in structural composition, and that insufficient consideration has been given to the possible significance of functional variations, such as arteriospasm, which may precede structural alterations. Unfortunately it is difficult in the living subject to obtain satisfactory evidence of vasoconstriction in the calibre of the precapillary arterioles of the retina, so that any attempt to bridge the gap between functional and structural aberrations can have at the present time no direct factual support. Biomicroscopical studies of the fundus will no doubt prove of value in establishing the importance of functional alterations, and it is not improbable that, as in the case of the choroidal circulation, methods will be devised by which the living retinae of experimental animals may be exposed and studied microscopically.

Meanwhile it would be unwise to speculate further upon the possible

relationship between structure and function in the genesis of diabetic retinopathy, but it may be of value to enumerate a few points of importance.

First, it would seem feasible that prolonged vasoconstriction, by increasing the density of the vascular wall, may hinder adequate movement of the tissue fluids within the vessel wall itself, reducing processes of diffusion and thus interfering with oxygenation and nutrition and resulting in intramural hyaline degeneration; a process which has been called "vasoconstrictive ischaemic anoxaemia" (Hueper, 1944). Indeed, there is considerable experimental evidence to show that prolonged vascular spasm may produce degenerative and proliferative changes in the vessel wall; thus arteriosclerotic and arteriolonecrotic changes have been induced by the repeated injection of adrenaline (Josué, 1905; Waterman, 1908; Stief and Tokay, 1935), and other vasotonic agents such as nicotine (Hueper, 1943), tyramine (Duff, Hamilton, and Magner, 1939), and ergotine (Yater and Cahill, 1936).

Secondly, it has been shown (Chambers and Zweifach, 1944), that the blood supply to the peripheral vascular bed is adjusted to the needs of the tissue by alternate contraction and relaxation of muscle cells in the metarterioles and precapillaries (the "metarteriolar musculature" and "precapillary sphincters") in response to vasoconstrictor impulses and the action of accumulated metabolites. The participation of humoral factors has latterly been held to be important; Shorr and his co-workers (Shorr, Zweifach, and Furchgott, 1945, 1948; Shorr, Zweifach, Furchgott, and Baez, 1947; Shorr, 1948) have shown that this peripheral circulatory mechanism is largely controlled by two circulating vasotrophic factors, which they have named "vaso-excitor material" (V.E.M.) and "vaso-depressor material" (V.D.M.). More recently Ashton and Cook (1952) have shown that cotrisone is able to exert a vasoconstrictive action, which may also operate through the metarteriolar musculature and precapillary sphincters. This finding is of particular interest in view of the possible relation of some adrenal cortical functions to retinopathy and glomerulosclerosis in diabetes (Becker, 1952).

This new concept of the peripheral circulation, however, has not so far been shown to apply to the retinal circulation, although Loewenstein (1946) claimed to have demonstrated "cushion cells" at the point of bifurcation of arterioles, which he believed might serve to regulate the retinal arterial blood supply. If such a controlling mechanism should eventually be proved to exist in the retina, it would at once provide an explanation for the site of election of the arterial constrictions in the diabetic retina, situated as they are in the terminal arterioles and precapillaries. It would then be necessary to enquire into the possibility of some pathological imbalance in the diabetic between the humoral factors responsible for maintaining normal vascular tone, or to seek an abnormal vasoconstrictive substance in the circulation.

The demonstration of such a mechanism would seriously challenge the hypothesis that diabetic retinopathy is primarily a venous disease, for, theoretically, the venous changes of dilatation and micro-aneurysm formation

might arise from prolonged anoxia through a chronically inadequate arterial blood supply. In view, however, of the absence of such venous changes in arteriosclerosis and benign hypertension, it is very unlikely that arteriospasm could alone be responsible for the early stages of diabetic retinopathy. Nevertheless, it is to be remembered that the concept of a primary venous involvement is unsatisfactory in a number of ways. It finds no parallel in the extra-ocular vascular complications; no progress has been made in the identification of a toxin having a selective action on the venous side, as postulated by Ballantyne (1946), while substances with a potentially toxic action, known to circulate in the blood of diabetics, have not, so far, been clearly incriminated (Hanum, 1938). These negative findings, while not excluding the theory of a toxin with a directly selective action upon the venous endothelium, turn attention towards the possibility that the venous involvement may be a secondary effect of a functional interference with the circulation in an earlier part of its course. In this connection it is interesting to note that Cholst and others (1952) have recently shown that in many diabetics, *without ophthalmoscopic evidence of significant retinal arteriosclerosis*, there is an inability of the retinal vessels to dilate maximally after intravenous injections of Priscoline. This defect was accompanied by perimacular exudation. A similar finding was obtained by Handelsman and others (1952) in the skin vessels of the toes of diabetics, which failed to dilate maximally, as measured by skin temperature readings, after Priscoline administration. Megibow and others (1949) from their studies on the vessels of the extremities of diabetic subjects, concluded that the vascular damage is localized in the arterioles and capillaries.

Finally, if arteriolar constriction should prove to be a factor of aetiological significance in diabetic retinopathy, a unifying concept for the pathology of all the vascular retinopathies could be propounded, in which the severity, extent, acuteness of onset, and duration of the arterial changes would be the most important points in accounting for the development of the differing ophthalmoscopic and microscopical pictures.

These arguments, however, are not advanced in support of any new hypothesis and are no more than reflections upon the possible significance of arteriolar involvement in diabetic retinopathy. It is realized that they include only a few of the many factors to be taken into account in considering the complex problem of the pathogenesis of the disease, but they may serve to emphasize the danger of restricting thought to the interpretation of changes in structure to the exclusion of function.

Summary

Changes in the arterioles in diabetic retinopathy have been studied in retinae removed *post mortem*, injected with Indian ink, and stained by the periodic acid-Schiff method. It has been found that the retinal arterioles are subject to a type of hyaline degeneration as severe as that which occurs

elsewhere in the vascular system in diabetes. Hyalinization in the terminal arterioles and precapillary vessels of the retina leads to narrowing and gradual occlusion of their lumina, resulting in atrophy of the arterial side of the capillary bed and the formation of new channels in the venous side of the capillary network. Complete obliteration of the arterioles eventually leads to the disappearance of the entire capillary bed.

This picture, resulting from arterial disease in the diabetic retina, has not so far been paralleled in numerous examinations of injected retinæ from non-diabetic cases with hypertension or arteriosclerosis. It is probable therefore, that the severe degree of arteriolar involvement should not be regarded merely as a super-added hypertensive complication, but as a characteristic late development of the diabetic process itself.

The arterial obliteration may be due to a complicating sclerosis unrelated to the genesis of the early stages of the retinopathy, or it may represent a structural change in vessels previously involved in chronic functional disorder. The probability and significance of these alternatives are discussed

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