PERICYTES IN DIABETIC RETINOPATHY*

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CONSIDERABLE significance has recently been attached to the role of intramural pericytes in the pathology of retinal vessels, and their specific injury is believed to have fundamental importance in the genesis of diabetic retinopathy. These stimulating new thoughts about an old and difficult problem have been advanced by Cogan and his co-workers (Cogan, Toussaint, and Kuwabara, 1961; Kuwabara and Cogan, 1963a) and merit careful consideration. In the present study the pathology of diabetic retinopathy has therefore been re-examined with their reports in mind, to determine how far their conclusions may be supported. No attempt will be made to review the large and controversial literature dealing with intramural pericytes; this paper is concerned only with the significance of their alteration in diabetic retinopathy.

Material and Methods

Thirty retinae digested with pepsin–trypsin (Ashton, 1952, 1963) from 25 cases of diabetic retinopathy and cortical vessels from 25 brains of patients with severe diabetic retinopathy were studied.

These observations were compared with studies of retinae from central venous occlusion with or without glaucoma, polycythaemia, cyanosis, and macroglobulinaemia, and normal, or at least non-diabetic, retinal and cerebral specimens from man, monkey, pig, cat, rabbit, and rat. The cortical vessels were obtained using Ashton's shake method (Ashton, 1949, 1963; Ashton, Kok, and Foulds, 1963) in fixed brains, and with the acid water technique (Oliveira, 1964)* in the fresh brains. Periodic acid-Schiff (PAS) and haematoxylin, or haematoxylin and eosin were used as the routine stains.

Observation and Comments

In this paper the term "intramural pericyte" is used to describe the cells embedded in the basement membrane of the small vessels (Ashton and Oliveira, 1966), which in the retina Kuwabara and Cogan (1960) first thought to be glial cells. Later, Kuwabara, Carroll, and Cogan (1961) called them "mural cells". They have made a number of statements referring to the distribution and significance of these cells, as follows:

* Received for publication June 18, 1965.

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(1) "We do not know that they exist in any vessels other than in the retina." "At the present we think they are unique to the retina." (Cogan, 1963.)

(2) "They are preferentially lost in diabetes from those vessels that give rise to microaneurysms and from vessels over a considerable area in the surrounding neighbourhood." (Cogan and others, 1961.) "The one condition—and the only one to our knowledge—in which mural cells are preferentially lost is diabetes." (Kuwabara and Cogan, 1963a.)

(3) "Microaneurysms are derived from vessels which have selectively lost their mural cells." "The minimal outpouchings or microaneurysms begin at those places in the capillary wall which have been weakened by loss of the mural cells." (Cogan and others, 1961.) "Almost 100 per cent. of the cases of diabetic aneurysms showed disappearance of the nucleus of the mural cells." (Kuwabara and Cogan, 1963b.)

(4) "Mural cells also appear to be associated with the control of neovasculogenesis in the retina. New vessel formation in the retina ceases in the foetus as mural cells appear, and conversely, neovasculogenesis occurs in the adult only after the mural cells have disappeared." (Kuwabara and Cogan, 1963a.) "Mural cells are probably contractile and control blood flow through the vessels." (Cogan, 1962.) "Specifically, loss of mural cells from capillaries seems to increase significantly the frequency with which red blood cells are found in these capillaries." "The mural cells may well be the crucial factor in excluding red blood cells from most of the capillaries most of the time." "Loss of mural cells results in pathologic arteriovenous shunts." (Kuwabara and Cogan, 1963a.)

In the present study of the subject these concepts cannot be shared and the above questions will now be considered in order.

(1) Are Intramural Pericytes confined to Retinal Vessels?

The problem of the nature of the cells first described by Rouget (1873, 1874, 1879) in the capillaries of young tadpoles and in the hyaloid membrane of the frog's eye, later named Rouget cells by Vimtrup (1922), and afterwards called pericytes by Zimmermann (1923), will not be discussed here. The relation of these cells with those which are described inside the basement membrane has been dealt with previously (Ashton and Oliveira, 1966). In this section only their distribution will be considered.

Cells which had the same appearance and the same fundamental characteristic of location inside the basement membrane as those thought by Cogan and co-workers (1961) to be confined to the retinal vessels, were first described in electron microscopical studies of the cerebral cortex (Farquhar and Hartmann, 1956), and later in similar studies of the retina (Maeda, 1958, 1959); of the iris (Ikui, Mimatsu, Maeda, and Tomita, 1960); of the cremaster muscle (Majno and Palade, 1961); of the choroid (Hogan and Feeney, 1961); of the ciliary body (Taniguchi, 1962); of the bat pancreas (Fawcett, 1963); of connective tissue (Movat and Fernando, 1964); and of skeletal muscle (Freeman, 1964). Other workers have since confirmed these observations. I have seen them in the retina, iris, conjunctiva, and brain by electron microscopy, and in the retina, conjunctiva, optic nerve, brain, meninges, skin, and peritoneum by light microscopy.

FIG. 1.—Two different patterns can be seen together in the equatorial zone of one diabetic eye. Above there are predominantly endothelial cells with remarkable degeneration of the intramural pericytes. Below a completely opposite pattern occurs. In both areas, however, micro-aneurysms can be seen. Pepsin-trypsin digest preparation. PAS and haematoxylin. $\times 66$.

A

B

FIG. 2.—A, B, Two completely different reactive patterns situated near the equator equidistant from the disc in the same retina from a case of diabetic retinopathy. In Fig. 2A intramural pericytes are selectively absent. In Fig. 2B they are present in practically normal numbers, but the endothelial cells have disappeared. Which of the two patterns can be said to be more representative? Pepsin-trypsin digest preparation. PAS and haematoxylin. $\times 66$.

The nuclei of these cells present as conspicuous spherical shapes in vessels in the nervous tissue, while in other tissues they are more elongated. In all circumstances, however, they are located inside a split of the basement membrane and show dense staining. By measuring the distance between the nuclei of these cells in capillaries of comparable calibre a slightly greater number of intramural pericytes was found in the human cerebral cortex than in the retina. In one case in the brain and the retina of an 82-year-old man, the average distance between the pericytes (in capillaries of 7 to 9 μ in diameter) was 72 μ in the retina, and 50 μ in the brain in a total of 200 measurements. The statement that intramural pericytes are confined to the retinal vessels cannot, therefore, be supported.

(2) Is Selective Degeneration of the Intramural Pericytes a Specific Process in Diabetic Retinopathy?

In the diabetic retinae studied, neither a constant nor dominant pattern of reaction of the intramural pericytes could be found. In the same retina, between the disc and ora serrata, and even in the same annular zone (Fig. 1), completely different reactive patterns were frequently observed in adjacent areas. Thus, if in some of these areas with more or less well-preserved endothelial cells, the intramural pericytes were selectively lost or degenerate (Fig. 2A), those areas where the contrary happened seemed to be even more frequent (Figs 2B and 3). In some retinae this pattern was seen in an extreme form, the endothelial cells being almost entirely absent, while the intramural pericytes, although in a more or less advanced stage of degeneration, could still be seen in a great number. The co-existence of both these patterns of reaction in diabetic retinopathy has already been emphasized by Ashton (1963).

Besides this process of degeneration of the intramural pericytes which may end with the appearance of minute outpouchings of the vessel wall, first described by

FIG. 3.—Area around the disc in a case of diabetic retinopathy in a 52-year-old patient. The endothelial cells have disappeared almost entirely and the intramural pericytes remain in considerable numbers. Note the presence of some microaneurysms. Pepsin-trypsin digest preparation. PAS and haematoxylin. $\times 52$.

FIG. 4.—High-power picture showing different stages of the migration movements of intramural pericytes. Pepsin-trypsin digest preparation. PAS and haematoxylin. $\times 666$.

FIG. 5.—Intense migratory activity of the intramural pericytes in a vessel from which endothelial cells have disappeared. Pepsin-trypsin digest preparation. PAS and haematoxylin. $\times 666$.

Cogan and others (1961) under the name of "mural cell ghosts", I observed another type of alteration of these cells not previously recorded, namely, migration, which like "ghost" formation I believe to be equally non-specific. It appears that the intramural pericytes, normally enclosed in the basement membrane, under unfavourable conditions (although more protected than the endothelial cells), may migrate like endothelial cells and follow them along the lumen (Figs 4 and 5). At least, this is my interpretation of the transformations undergone by nuclei, which in normal conditions present a conspicuous spherical shape in retina and brain. This phenomenon of migration of intramural pericytes could be seen not only in diabetic retinopathy but also in cases of thrombosis of the central retinal vein with or without glaucoma, in polycythaemia, and in macroglobulinaemia. Very rarely was it seen in normal eyes or in the brain.

Although degeneration of the intramural pericytes is a striking feature of diabetic retinopathy, it cannot be specifically related to diabetes, even when their loss is selective. They were found to be selectively degenerate in macroglobulinaemia (Ashton, Kok, and Foulds, 1963) and the same could be seen in the cases of cyanosis and polycythaemia studied (Figs 6 and 7). Moreover, "ghost" formations or degenerated intramural pericytes were not found in diabetic brains even when they were present in the retinae from the same case.

While intramural pericyte degeneration may be peculiar to the retina, and more pronounced in diabetes than in other conditions, it is concluded that such alteration is quite probably a non-specific change. Unless there is a particular type of pericyte degeneration in diabetes, my findings do not support the view that their alteration is peculiar to diabetic retinopathy.

FIG. 6.—Retinal vessels from a case of cyanosis showing remarkable selective loss of intramural pericytes. Pepsin-trypsin digest preparation. PAS and haematoxylin. $\times 72$.

FIG. 7.—A similar area of selective degeneration of intramural pericytes from a case of polycythaemia. This is comparable to the picture found in some areas in diabetic retinopathy. Pepsin-trypsin digest preparation. PAS and haematoxylin. $\times 50$.

(3) Are the "Ghosts" of Intramural Pericytes responsible for Micro-aneurysms?

Micro-aneurysms are perhaps the commonest lesions of the retinal vessels. They are far from being peculiar to diabetic retinopathy, and their features in this disease do not present any specific character when compared with those of the non-diabetic (Ashton, 1963). Whereas it is true that micro-aneurysms can be found more abundantly in areas where intramural pericytes are, or appear to be, selectively absent, they can be seen also in areas where intramural pericytes are the dominant remaining cells (Figs 3 and 8). Moreover, intramural pericytes of normal appearance

FIG. 8.—A case of diabetic retinopathy showing some micro-aneurysms in one area from which the endothelial cells have disappeared almost completely and in which intramural pericytes are still present. Pepsin-trypsin digest preparation. PAS and haematoxylin. $\times 72$.

FIG. 9

FIG. 10

FIG. 11

FIG. 9.—High-power photomicrograph showing that micro-aneurysms in diabetic retinopathy can appear in capillaries in which intramural pericytes are present in normal numbers. Note that there is a pericyte (P) in the wall of one micro-aneurysm, and another at the edge of the other aneurysm. The ghost cell (G) is not associated with an aneurysm.

FIG. 10.—In this case, from another diabetic retina, the micro-aneurysm was formed in the capillary wall opposite to an intramural pericyte.

FIG. 11.—This third high-power view, also in a diabetic retina, shows a "ghost" of a degenerated pericyte nucleus at one edge of the micro-aneurysm (G); this aneurysm, however, did not develop in the direction of the "ghost" cell. The two darkly stained nuclei in the micro-aneurysm are probably intramural pericytes.

are not infrequently seen near or even in the wall of the micro-aneurysms (Figs 9 and 10) and, conversely, degenerate "ghost" pericytes without aneurysmal formation are a common finding. On the other hand, micro-aneurysms could be seen in the immediate neighbourhood of "ghosts", suggesting that intramural pericyte degeneration does not provide an especially vulnerable focus (Fig. 11). Ashton, Kok, and Foulds (1963) emphasized this absence of relationship also in macroglobulinaemia, and in the cases of polycythaemia here observed—despite widespread degeneration of intramural pericytes and "ghost" formation—few micro-aneurysms could be seen. Thus, throughout these observations, no significant correlation could be established between intramural pericyte "ghosts" and micro-aneurysm formation.

(4) Do Intramural Pericytes inhibit Neovasculogenesis?

In the present observations the only truly dominant and constant phenomenon concerning intramural pericytes was their scarcity or entire absence in vessels where endothelial proliferation had occurred.

This, however, does not necessarily mean that intramural pericytes and endothelial proliferation, or neovasculogenesis, are incompatible, for it is more probable that the pericytes here behave like endothelial cells in reacting to the same stimulating factors by proliferation. Thus the vessels return to their embryonal appearance where the growing endothelial tubes show little differentiation between endothelial cells and pericytes (Shakib and Oliveira, 1966). This explains why in adult proliferating vessels studied by light microscopy, intramural pericytes seem to be absent, as in the embryonal stages.

(5) What is the Role of the Intramural Pericytes in Circulatory Control?

In diabetic retinopathy Kuwabara and Cogan (1963a) observed that loss of pericytes from capillaries seemed to increase significantly the frequency with which red blood cells could be found within them, and according to the thesis advanced by Thuránszky (1957) that most of the retinal capillaries exclude red blood cells, they attributed their findings to the loss of the normal contractile function of the intramural pericytes. According to their interpretation, the specific loss of pericytes resulted in the formation of shunts and consequent ischaemia of adjacent capillaries and was thus of great importance in the pathogenesis of diabetic retinopathy. Against this thesis, however, we have seen in some retinae large areas of the capillary net filled with red corpuscles where intramural pericytes were almost the only cells remaining and, although they may be functionally impaired, we have no evidence that this is so. Moreover, even if it seems reasonable to infer that intramural pericytes may be contractile, this has never in fact been demonstrated; on the contrary, recent *in vivo* observations by Friedman, Smith, and Kuwabara (1964) showed that the microvascular bed of the retina is characterized by its steady flow with no spontaneous changes in capillary calibre. The concept that the capillary pathology of diabetic retinopathy is dependent upon haemodynamic changes resulting from selective degeneration of the intramural pericytes is therefore unsubstantiated; indeed, the reverse relationship, that pericyte degeneration results from haemodynamic changes, may well be the case.

Conclusions

Intramural pericytes are described in this work as cells embedded in the capillary basement membrane. Cells with this fundamental characteristic of location were shown in several tissues, and are thought to be present in the capillary basement membrane throughout most of the body.

Degeneration of intramural pericytes could not be specifically related to diabetes even when their loss was selective. They were found to be selectively degenerate in the retina in other conditions, such as macroglobulinaemia, cyanosis, and polycythaemia. It is believed that under unfavourable conditions intramural pericytes would react in two ways: by migrating into the lumen, or by degenerating *in situ*, ultimately forming "ghost cells". Neither of these processes shows evidence of being specific for diabetic retinopathy.

No significant relationship between "ghost cells" and micro-aneurysm formation could be established, nor could any specific implication of the pathology of intramural pericytes in the pathogenesis of diabetic retinopathy be found.

In the presence of unknown stimulating factors it is believed that intramural pericytes, like endothelial cells, can proliferate. The theory of a relationship between these cells and the inhibition of neovasclogenesis could not be supported.

At the present time it is believed that the function and pathology of intramural pericytes remain unknown, and the concept that the capillary pathology of diabetic retinopathy is dependent upon haemodynamic changes resulting from selective degeneration of the intramural pericytes is unsubstantiated.

Summary

Thirty digested retinae and isolated vessels from 25 brains of patients with a severe degree of diabetic retinopathy were examined.

The findings were compared with those made on digested retinae from occlusion of the central retinal vein, with or without glaucoma, from polycythaemia, from cyanosis, from macroglobulinaemia, and from normal human eyes. Retinal and brain vessels from the monkey, pig, cat, rabbit, and rat were also examined.

I should like to thank Prof. Norman Ashton for his constant encouragement, advice, and enlightening interest in this work. It is a pleasure to record my thanks to Mr. A. McNeil, Mr. J. Peacock, and Miss N. A. Yarkoff for technical assistance, and to Miss E. FitzGerald for secretarial help.

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