the view that there are immunological differences between serum hepatitis and infectious hepatitis.

Finally, the presence of virus in the face of circulating antibody adds more weight to previous observations that antigen-antibody complexes may play a part in some forms of hepatitis (Almeida and Waterson, 1969). This again implies that the state of the antigen and the presence of antibody could be related to different host responses and may be a basis for the clinical and laboratory differences often observed between infectious and serum hepatitis.

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Degeneration of Intramural Pericytes in Diabetic Retinopathy

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Summary: Retinal pericyte degeneration, a usual finding in diabetic retinopathy, has been shown to be a change probably peculiar to the retina rather than part of a more widespread angiopathy. Thus in a patient with diabetic retinopathy the characteristic eosinophilic degeneration of the pericyte nuclei ceased at the optic disc and could not be found in vessels of the optic nerves and brain.

Introduction

Kuwabara and Cogan (1960) introduced a trypsin digestion technique whereby the retinal vessels could be isolated from retinal tissue. Among the many new findings found by this method was the presence of cells within the basement membrane of the capillary walls. Kuwabara and Cogan (1963) considered these cells to be peculiar to the retinal circulation and had named them "mural cells" (Kuwabara, Carroll, and Cogan, 1961). They believed that these cells were probably concerned in the control of capillary blood flow and, since they later discovered that these cells were selectively injured in diabetic retinopathy, concluded that they were of significance in the pathogenesis of this complication (Cogan, Toussaint, and Kuwabara, 1961). It was subsequently pointed out (Ashton and Oliveira, 1966) that these cells correspond to the long-recognized and widely distributed capillary "pericyte," one form of which had also, since the introduction of the electron microscope, been shown to lie within the basement membrane (Farquhar and Hartmann, 1956; and others). To describe their situation more accurately, to prevent confusion in the future, and to retain association with the original work of Cogan and his colleagues, it was suggested that they now be called "intramural pericytes" as distinct from "extramural pericytes" lying outside the basement membrane and that the name "pericyte" should be the general term (Ashton and Oliveira, 1966).

In diabetic retinopathy Cogan *et al.* (1961) have reported a selective loss of intramural pericytes from the capillaries which retained their endothelium, and these were replaced with "ghost cells." According to our own observations it is not so much the selective absence of intramural pericytes which is so characteristic of diabetes, for this is a common finding in scattered areas of many non-diabetic retinae examined post mortem, but the presence of the "ghost cells" they described in digest preparations stained with P.A.S. (periodic-acid-Schiff stain). If the digests are stained with haematoxylin and eosin many of these "ghost cells" appear intensely eosinophilic and the picture is much more striking than the negative staining with P.A.S. While we have not found this eosinophilic degenerative change to be entirely specific for diabetes, if it is pronounced and widespread it is almost diagnostic of diabetic retinopathy.

Kuwabara *et al.* (1961) postulated that this degenerative change provided weak points on the capillary walls predisposing to aneurysmal dilatations and was responsible for the formation of arteriovenous shunts through the vascular bed, resulting in drainage and closure of the surrounding capillaries (Cogan *et al.*, 1961). The loss of the intramural pericyte appeared, therefore, to be a factor of crucial importance,

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which might quite well be due to some circulatory disturbance in the diabetic state, and one of the many questions that naturally arise is whether intramural pericytes elsewhere in the body degenerate in a similar way.

We have confined our attention particularly to the intramural pericytes of the vessels of the optic nerve and brain, which are closely comparable to those of the retina, not only in their cellular structure but also in possessing similar blood barriers (Ashton, 1965a, 1965b). Earlier studies from this department involving the examination at necropsy of the brains from 26 diabetics with retinopathy suggested that the cerebral vessels do not, in fact, share in this degenerative process (Ashton, 1963; Oliveira, 1966). These conclusions, however, were based on a comparison of cerebral capillaries isolated by shake (Ashton, 1949) or acid-water techniques (Oliveira, 1964) with retinal capillaries exposed by a proteolytic enzyme digestion technique. Clearly these findings are not fully acceptable unless they can be substantiated by studies using comparable methods; the work reported in the present paper remedies this defect.

Material and Methods

The eyes, with their attached optic nerves, and specimens of cerebral cortex from the frontal, parietal, and occipital lobes were obtained at necropsy from an 80-year-old woman who had been a known "well-controlled" diabetic for 10 years. Simple non-proliferative retinopathy had been present for at least 18 months, its recognition only being possible following cataract extraction.

The left globe was opened in the unfixed state in a coronal plane through the ora serrata and examined under a dissecting microscope. After removal of the vitreous the retina was separated from the choroid and divided into two portions, one for pepsin-trypsin digestion and the other for acid-shake technique. The right eye was fixed in formol saline and retained for other studies. Duplicate 1-mm. thick sections from each of the brain specimens were taken, one being set aside for digestion and one for acid-shake.

The intraorbital optic nerve was divided longitudinally, the two portions being treated by the two separate techniques.

The acid-shake technique was performed by placing the specimens in separate flasks containing distilled water acidulated to *p*H 2·3 with hydrochloric acid. The flasks were attached to a Griffin flask shaker and agitated simultaneously for 16 hours. The specimens were then washed in distilled water, teased out on a glass slide to reveal the capillaries, and stained with haematoxylin and eosin.

The specimens for digestion were fixed in 10% formol saline as soon as they had been separated from the other specimens, and each was treated by the pepsin-trypsin technique (Ashton, 1963). The specimens were teased out on glass slides and stained with haematoxylin and eosin.

Results

The opened globe viewed under the dissecting microscope showed multiple small cotton-wool spots in the posterior fundus and several capillary microaneurysms.

Examination of the enzyme-digested and shake retinal specimens showed a selective degeneration of the pericyte nuclei in capillaries from the posterior fundus, characterized by eosinophilic staining of the nuclei and in some instances complete loss of nuclear staining (Figs. 1 and 2). The capillary endothelium was, by comparison, relatively intact and, though in some areas capillaries which had lost both their pericyte and endothelial nuclei were seen, there was no sign of eosinophilic degeneration in the endothelium. No

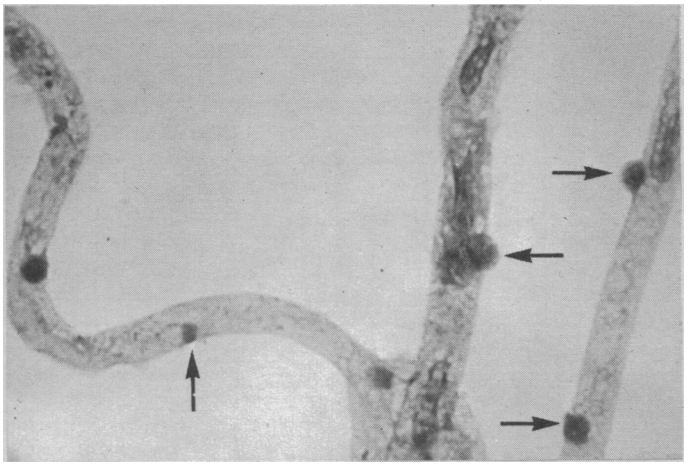

FIG. 1.—Retinal capillaries isolated by pepsin-trypsin digestion. There is widespread eosinophilic degeneration of intramural pericytes (arrows). The palely staining endothelial cells were basophilic. (H. & E. ×486.)

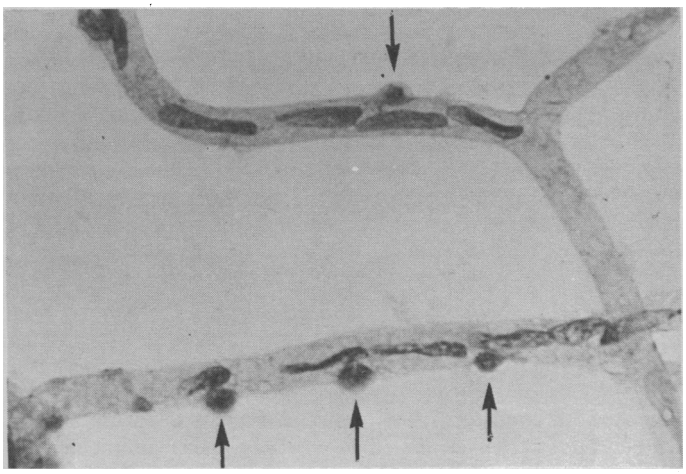

FIG. 2.—Retinal capillaries isolated by shaking show a similar picture of eosinophilic degeneration of pericytes (arrows) with normal basophilic staining of endothelial cells. (H. & E. ×486.)

difference in either number or staining reaction of the pericyte nuclei was observed between the digested and acid-shake specimens.

In contrast to these findings in the retina, examination of capillaries from the cerebral cortex specimens, prepared by acid-shake technique, and from the optic nerve, prepared by both digestion and acid-shake techniques, failed to show any discernible change in either the pericyte or the endothelial cell nuclei (Figs. 3 and 4). Satisfactory specimens could not be obtained by pepsin–trypsin digestion of the formol-fixed brain specimens.

The presence or absence of eosinophilic degeneration in the pericyte nuclei in the different tissue treated by the two methods is summarized in the Table.

Eosinophilic Degeneration

	Acid-shake			Pepsin–Trypsin Digestion		
	Retina	Brain	Optic Nerve	Retina	Brain	Optic Nerve
Eosinophilic intramural pericytes	Present	Absent	Absent	Present	Satisfactory specimens unobtainable	Absent

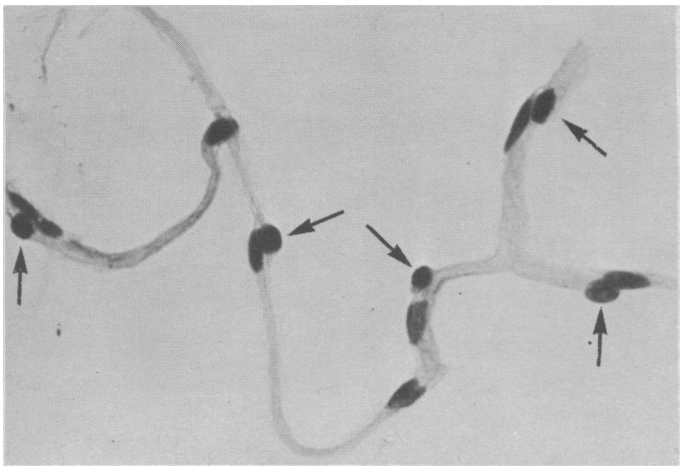

FIG. 3.—Cerebral capillaries isolated by shaking show normal basophilic staining of both pericytes (arrows) and endothelial cells. (H. & E. ×486.)

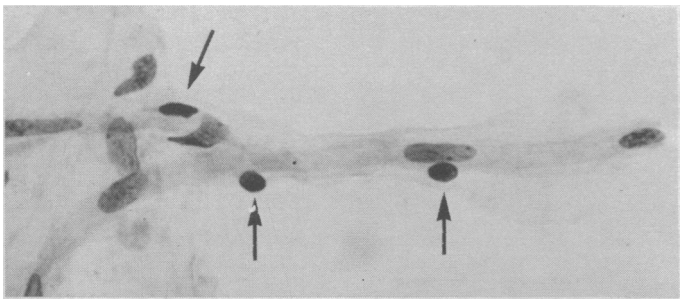

FIG. 4.—Capillary isolated from optic nerve by shaking shows normal appearance of pericytes (arrows) and endothelial cells. (H. & E. ×486.)

Comment

The foregoing studies, using comparable methods of preparation, confirm earlier reports from this department that degenerative changes in the capillary pericyte nuclei of diabetic patients are probably peculiar to the retina and not part of a generalized disturbance in the vessels of the nervous system (Ashton, 1949; Oliveira, 1966). Furthermore, the abrupt appearance of such pericyte degeneration at the level of the optic disc would suggest that local retinal factors are of major importance in its pathogenesis and, consequently, that its absence from vessels of the central nervous system may well be true for capillaries elsewhere. The significance of the claim by Yodaiken *et al.* (1967) that there are ultrastructural signs of pericyte degeneration in skin capillaries of diabetics is uncertain and must await more convincing evidence, especially as it is not confirmed by other studies in this field (Friederici *et al.*, 1966). It is evident that the eosinophilia seen in such cells in diabetic retinopathy is not an artifact caused by enzyme digestion. It would appear from preliminary studies carried out in this department that it represents a specific loss or degradation of nucleoprotein, which proceeds to a situation wherein the degenerate pericyte nucleus is merely represented by an unstained ghost outline (unpublished data).

While selective pericyte degeneration in the presence of intact endothelial cells is characteristic of diabetic retinopathy it is not specific for this condition, since similar changes have been demonstrated in a variety of conditions such as macroglobulinaemia (Ashton *et al.*, 1963), myelomatosis (Ashton, 1965b), cyanosis and polycythaemia (Oliveira, 1966), and occasionally in patients dying of cerebral haemorrhage or coronary thrombosis (Ashton, 1969). Nevertheless, the extent of the intramural pericyte loss is very much less in non-diabetic retinopathy. The precise reason for the selective involvement of the intramural pericyte in these conditions, wherein impaired retinal circulation is a common factor, remains speculative.

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