Reticulin fibres in relation to retinal vessels

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SUMMARY Argyrophilic perivascular and intervascular fibres in the mammalian retina are shown by specific antireticulin immunofluorescence to consist of reticulin. The possible significance of these findings is briefly discussed.

Some recent studies in the retina of man and animals have reported the presence of perivascular and intervascular fibres which, by reason of their argyrophilia, ultrastructure, and enzymatic digestion were believed to consist of reticulin (Daicker, 1971a, b; Ashton and Tripathi, 1975). The latter authors, however, pointed out the unreliability of these criteria and suggested that the problem might be resolved by the use of specific immunofluorescent staining of reticulin.

An immunohistochemical method for demonstrating reticulin fibres in tissues has recently been described (Seah *et al.*, 1971). Human sera containing reticulin antibodies were found to stain reticulin fibres in the rat liver, stomach, and kidney, and the patterns resembled very closely the silver impregnation staining technique. The immunofluorescence was not impaired, and was even enhanced by pre-treatment with collagenase but was abolished by periodate, and the authors were convinced that the fibres were in fact reticulin. Moreover, a considerable advance was made by Pras and Glynn (1973), who recently extracted from reticulin by a water dispersion method a noncollagenous protein, the antibodies of which showed specific antireticulin activity by immunofluorescent techniques. We have employed both these methods of investigation in the retinas of monkeys and rats and now report our findings.

Material and methods

RETICULIN ANTISERA

The specific antisera used in the present study were obtained from patients suffering from either uveitis or coeliac disease, conditions known to be associated with the presence of antireticulin anti-

bodies (Seah *et al.*, 1971; Rahi *et al.*, 1976) or else prepared in this laboratory in New Zealand albino rabbits according to the following schedule: 4 mg of a noncollagenous component of reticulin protein prepared according to the method of Pras and Glynn (1973)*, was ultrasonically dispersed in 2 ml of distilled water, and emulsified in equal volumes of Freund's complete adjuvant and injected intramuscularly into the animals at 4 different sites. The injection was repeated twice at 2 weeks and 4 weeks after the initial dose, and the animal was bled 1 week after the last injection. The serum was stored in aliquots at -20°C .

IMMUNOFLUORESCENCE TEST

Frozen sections of a composite block consisting of the liver, kidney, diaphragm, and stomach of a rat were used to test the specificity of the antisera. Frozen sections of eyes from rats and monkeys and capillaries isolated from rat retinas by the acid water technique were stained to demonstrate the perivascular and intervascular reticular fibres. A standard indirect immunofluorescent staining technique was used. Fluorescein labelled antihuman and antirabbit immunoglobulins were obtained from the Wellcome Laboratories. A Zeiss epifluorescence microscope equipped with a fluorescein isothiocyanate (FITC) interference filter was used to examine the stained sections.

Findings

The reticulin antisera obtained from patients and from immunised rabbits gave typical staining patterns (Rizzetto and Doniach, 1973; Williamson *et al.*, 1976) in the liver, kidney, and stomach (Figs. 1, 2, and 3). The septal fibres within the connective tissue of the optic nerve stained intensely (Fig. 4).

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Fig. 1 *Indirect immunofluorescence test on rat liver. The antireticulin serum reacts with the reticulin fibres in the central portal vein (A) and the branches of hepatic artery (B) to produce a bright fluorescence. Unfixed cryostat section $\times 360$*

In the sections of the retina fluorescent staining was confined to the perivascular fibres and also outlined the vascular endothelium (Figs. 5, 6, 7, and 8). Isolated rat retinal vessels showed coarse longitudinal and branching fluorescent networks of perivascular fibres (Fig. 9). These appearances exactly corresponded to those demonstrated in silver-stained preparations (Ashton and Tripathi, 1975) and established the reticulin nature of these fibres (Fig. 10).

Discussion

The fact that reticulin exists in the retina in the form of perivascular networks and intercapillary strands and bridges is of academic interest and of pathological significance. There has for many years been controversy as to the biochemical nature of reticulin fibres and hesitancy in correlating their microscopical and ultrastructural properties, to the extent that the term 'reticulin' is generally avoided

Fig. 2 (left) *Peritubular reticulin fluorescence in a rat kidney demonstrated by the same technique as in Fig. 1 $\times 360$*

Fig. 3 (right) *Periglandular reticulin fluorescence in a rat stomach demonstrated by the same technique as Fig. 1 $\times 270$*

Fig. 4 *The septal reticulin fibres in the optic nerve of a monkey show bright fluorescence when treated with specific antiserum. Unfixed cryostat section $\times 175$*

by electron microscopists. Cervos-Navarro and Matakas (1975) claim that they were the first to describe the ultrastructure of reticulin in 1972

(Matakas and Cervos-Navarro, 1972). In view of the close anatomical relationship between basement membrane and reticulin, described in the retina by Ashton and Tripathi (1975), it is noteworthy that Cervos-Navarro and Matakas (1975) consider that

Fig. 5 *Cross-section of rat retina treated with antireticulin serum. Bright perivascular fluorescence was seen in the retinal vessels (right) and in the choroidal vessels (left). Indirect immunofluorescence test. Unfixed cryostat section $\times 360$*

Fig. 6 *Flat section of rat retina treated by the same technique as in Fig. 5. Bright fluorescence is present within the capillary walls $\times 360$*

Fig. 7 Positive fluorescence of reticulin fibres in the capillaries of the monkey retina (cross-section) seen partially in focus in a thick unfixed cryostat section $\times 360$

Fig. 8 Flat section of monkey retina showing fluorescence in a pericapillary reticulin network with strands extending into retina. Unfixed cryostat section $\times 480$

basement membrane is the substrate of reticulin, a correspondence first mentioned by Robb-Smith (1970). They emphasise that the fibrillar form of reticulin seems to be only a light-microscopical finding and possibly caused by formalin fixation in some instances. In the present study, however, we have demonstrated them by immunofluorescence in unfixed frozen sections; nevertheless, perivascular reticulin networks are certainly more readily demonstrated by silver staining in formalin fixed tissue.

The reticular fibres in the retinal vessels stained less brilliantly than those in the optic nerve, the liver, the kidneys, and the stomach. As the product of immunofluorescence, like any other chemical reaction, is generally directly proportional to the concentration of the reactants, this weak staining may be due to a relatively smaller amount of these fibres in the retina than in other tissues.

Since reticulin appears to exist in several different morphological (Vizioli *et al.*, 1974) and immuno-

Fig. 9 (A and B) Rat retina. Capillaries isolated by acid-water techniques show fluorescent networks around capillaries treated with specific antiserum. Unfixed $\times 1000$

Fig. 10 Retinal capillary from same retina as in Fig. 9 but post-fixed in formalin and stained by the Gomori method for reticulin. The fluorescent fibres now appear argyrophilic and show the typical network. The silver is probably deposited on the antigenic glycoprotein component of the reticulin $\times 1000$

logical forms (Rizzetto and Doniach, 1973; Boyd *et al.*, 1976), it is possible that the antiserum which we used in the present study was not strong enough to react with all the 'organ' and 'non-organ' specific reticular fibres in the retina.

There is now general agreement, however, that reticulin is a type of collagen protein rich in carbohydrate (Perez-Tamayo and Rojkind, 1973) in the form of polysaccharide side chains which effectively reduce the cross-linkage and so prevent aggregation into typical collagen fibres (Muir, 1973). It has only recently become clear that there are at least four genetically distinct types of mammalian collagen (Grant *et al.*, 1975; Rojkind and Martinez-Palomo, 1976). Type I, the conventional collagen found in cornea, skin, bone, and tendon, consists of two $\alpha 1(I)$ polypeptide chains and one $\alpha 2$ polypeptide chain. Type II, the type found in hyaline cartilage, consists of three $\alpha 1(II)$ polypeptide chains. Type III, found in small quantities in several connective tissues and in large amounts in the fetal skin and blood vessels, contains three $\alpha 1(III)$ polypeptide chains. It is now believed that reticulin fibres belong to this latter class of the collagen family but for reasons already referred to have failed to aggregate. Type IV, the fibrillar material found in basement membranes and the lens capsule, consists of three $\alpha 1(IV)$ polypeptide chains.

In previous papers the distribution of argyrophilic fibres in the normal retina has been demonstrated (Daicker, 1971; Ashton and Tripathi, 1975), and although it cannot be said that all such fibres are reticulin it is now clear that this may well be the

case, but the proliferating argyrophilic fibres found in retinal disease may have a glial component. This would seem unlikely, since it was shown in electron micrographs by Ashton and Tripathi (1975) that glia remained unstained with silver methenamine in contrast to the positive staining of adjacent proliferating perivascular fibres now identified as reticulin. It would appear, therefore, that some reactions in the diseased retina previously described as gliosis are rather to be regarded as collagenous in nature. But, since fibroblasts are not usually associated with such proliferations within the retina, the cellular source of the collagenous protein, whether from endothelial cells, muscle cells, pericytes, or all three, is unclear, while the frequent replacement in degenerate and senile vascular retinopathies of basement membrane and reticulin fibres by fibres ultrastructurally indistinguishable from conventional collagen (i.e., type I) has yet to be explained.

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