The proteins of the aqueous humour

J.P. DERNOUCHAMPS

University Eye Clinic, Université Catholique de Louvain, Belgium

Introduction

The incidence of uveitis in Belgium may be estimated at about 10 000 new cases per year. Of these, 25% become chronic (Perkins, 1961), in spite of treatment with corticosteroids, and frequently present complications such as ocular hypertony and cataract. The origin and the pathogenicity of most cases of uveitis (65–70%) are unknown (Perkins, 1968; Witmer, 1968).

The aqueous humour (AH), which washes the anterior uvea, may be used to study of factors involved in the etiopathogenicity of the uveitis. Analysis of this fluid has been found useful for semeiotic as well as physiopathological studies. For this reason, we decided to study the variation of the protein content of the AH and to carry out several serological tests on this fluid. In this monograph, our results will be related and discussed with regard to the data of the literature.

Analysis of AH has seldom given useful results in cases of uveitis. Viruses or bacteria are rarely detected, while cytological analysis only gives information on the intensity and the evolution of the uveal inflammation. The detection of neoplastic cells can sometimes confirm the diagnosis of intraocular tumour. The titration of specific antibodies gives more diagnostic information allowing us to determine the cause of uveitis in about 10% of cases. Immunochemical methods, in which we were interested, are sufficiently accurate for the specific determination of several proteins in a single sample of AH.

Chapter 1 will consider the analysis of the proteins of the AH taken from patients assumed to be normal or who were suffering from various diseases of the uvea. Since most of the AH proteins are derived from serum, we have stated some properties of the blood-aqueous barrier by using the information provided by quantitative determination of various proteins in AH and serum.

Reprint requests: J.P. Dernouchamps, avenue Xavier Henrard 20, B-1150 Brussels, Belgium.

List of abbreviations : Å : Angström; DNA : desoxyribonucleic acid; AH : aqueous humour; Ig : immunoglobulin; μ : micron; μg : microgram; μl : microliter; MW : molecular weight; TCA : trichloracetic acid.

Documenta Ophthalmologica 53, 193–248 (1982) 0012–4486/82/0533–0193 $8.40

These quantitative determinations have shown that the behaviour of transferrin, as is found in other biological systems, is unique. The concentration in the AH of this iron-binding glycoprotein is higher than can be expected from its molecular weight and serum concentration. Chapter 2 will be devoted to the description and the discussion of this phenomenon.

In chapter 3 we shall see that analysis of AH proteins is useful in clinical practice only in the case of the immunoglobulins. We shall also describe the results of the measurement of immune complexes and rheumatoid factor in the AH. These results, although limited, suggest that the detection of immune complexes or rheumatoid factor in the AH could have a prognostic value, especially in the development of cataracta complicata and sympathetic ophthalmia and, perhaps, of metastasis in cases of intraocular tumour.

Chapter 1

Analysis of the proteins of the aqueous humour

The AH contains little protein – less than 50 mg/100 ml – and its electrophoretic pattern is comparable with that of serum (von Sallmann and Moore, 1948; Esser et al., 1954; Wunderly et al., 1954; François et al., 1958). No protein specific for the AH has been found. Determination of the total protein content has been found by many authors to give more accurate information than that obtained from measurements of the Tyndall effect with a slit-lamp. Electrophoresis and immunoelectrophoresis have shown modifications of the gammaglobulins/albumin ratio in cases of uveitis (Wunderly and Cagianut, 1952; Cagianut, 1957; François and Rabaey, 1960; Yanagizawa, 1965; Faure et al., 1966; Michiels, 1968; Neupert and Lawrence, 1970; Stjernschantz et al., 1973).

The low AH/Serum concentration ratio – 1/250 for the total protein – and the poor transfer into the AH of macromolecules such as gum-arabic (MW: 250 000–300 000) (Appelmans and Michiels, 1947) or labelled albumin (Oeff, 1959), after intravenous injection into rabbits, indicate a substantial blood-aqueous barrier (BAB). The BAB is a barrier situated between blood and intercellular fluid, i.e. AH. Such barriers can be observed in the organism wherever there are capillaries. The nature of the exchanges through the BAB is responsible for the different properties of the AH, enabling it to carry out its triple function : tensional, owing to the equilibrium between inflow and outflow; optical, by its transparency, and metabolic. It is well known that the AH alone ensures the nutrition of the avascular lens.

Total protein content

To determine the total protein level we used the method of Meulemans (1960), which was recommended by Laterre (1965) for solutions with a total protein content in the range of 6 to 100 mg/100 ml. This method is based on precipi-

tation by trichloracetic acid (TCA) – used by Applemans and Michiels (1947) – but with a turbidimetric reading. It differs from other TCA methods in its low final concentration of precipitating agent (2.4 g/100 ml), to avoid flocculation which is a cause of error for turbidimetry. This error appears when the final concentration of TCA exceeds 5 g/100 ml.

The quantitative determination was carried out as follows. The absorbance at 450 nm in quartz microcells (Beckman, Fullerton, California) is read 10 min after the addition of 160 µl of a TCA solution at 3 g/100 ml to 40 µl of AH. We used as standards 7 dilutions of a solution of Labtrol (Dade, Miami, Florida) containing from 6 mg/100 ml to 96 mg/100 ml. The AH samples with a protein level higher than 100 mg/100 ml were diluted adequately and retested.

The mean total protein level of the AH in 12 patients suffering from senile, immature cataract, with no signs of irritation or previous inflammation, was 23.7 mg/100 ml (SD : 5.6 mg). We have taken this value as a standard for the protein level of the normal AH. Our results in rabbits (n = 40) were 25.9 mg/100 ml (SD : 15.6 mg) and in dogs (n = 7) 14.7 mg/100 ml (SD : 4.1 mg).

Our results in man were similar to those given by the micro-Kjeldahl method (Table 1), which is the method of reference, but takes longer to perform. They differed appreciably from values obtained by other methods. Since the selection of the patients was the same, these differences should be due only to the technical variations. Precipitations by TCA solution at 10 g/100 ml or by sulfosalicylic acid solution at 5 g/100 ml with nephelometric reading, or the fixation of Amido-black give lower values. This could be explained by the variations, according to the nature of the proteins, of the precipitating activity of sulfosalicylic acid (Bossak et al., 1949; Henry et al., 1956; Meulemans, 1960) and of the affinity of Amido-black (Hinsberg and Gleiss, 1950). The methods using the reagent of Folin-Ciocalteu give higher values, probably because of the presence of polypeptides and amino-acids (Laterre, 1965). Para-aminosalicylic acid, which is a tuberculostatic agent, and merthiolate, which is

Table 1. Total protein content of human aqueous humour (mg/100 ml)

Methods	References	Protein content (mean ± S.D.)
Micro-Kjeldahl	Duke-Elder (1927)	20.1
	Balavoine and Vuataz (1949)	23.1 ± 19.3 (n = 7)
Nephelometry		
with sulfosalicylic acid at 5 g/100 ml	Kronfeld (1941)	9.8 ± 2.9 (n = 14)
with trichloracetic acid at 10 g/100 ml	Alacrts (1948 a-b)	10
Amido-black	Wunderly et al. (1954)	14.8 ± 5.3 (n = 23)
	Remky (1954)	10
	Steiger et al. (1955)	12.7 (n = 38)
Folin-Ciocalteu	Heer (1957)	30.5 ± 4.1 (n = 20)
	Peretz and Tomasi (1961)	30 – 50
	Krause and Raunio (1970)	36 (n = 4)
Method of Meulemans	Dernouchamps and Michiels (1976)	23.7 ± 5.6 (n = 12)

a bactericide used as preserving agent for samples, may also interfere in these tests (Zondag and Van Boetselaer, 1960).

Total protein levels of the AH were increased from the normal 24 mg/100 ml to as much as 5 000 ml/100 ml in patients suffering from various ocular diseases (Fig. 1). An increasing in the total protein content of AH is generally considered to reflect the intensity of the inflammation but not to be useful for diagnosis. An exception to this is that values higher than 80 mg/100 ml effectively exclude the diagnosis of Fuchs' cyclitis.

Specific quantitative determination of proteins

The concentrations of various proteins were determined by single radial immunodiffusion (Mancini et al., 1965). If an antigen is allowed to diffuse radially from a well in a layer of antibody-containing agar, the area finally occupied by the precipitate is directly proportional to the amount of antigen and inversely proportional to the concentration of antibody (Fig. 2). This method

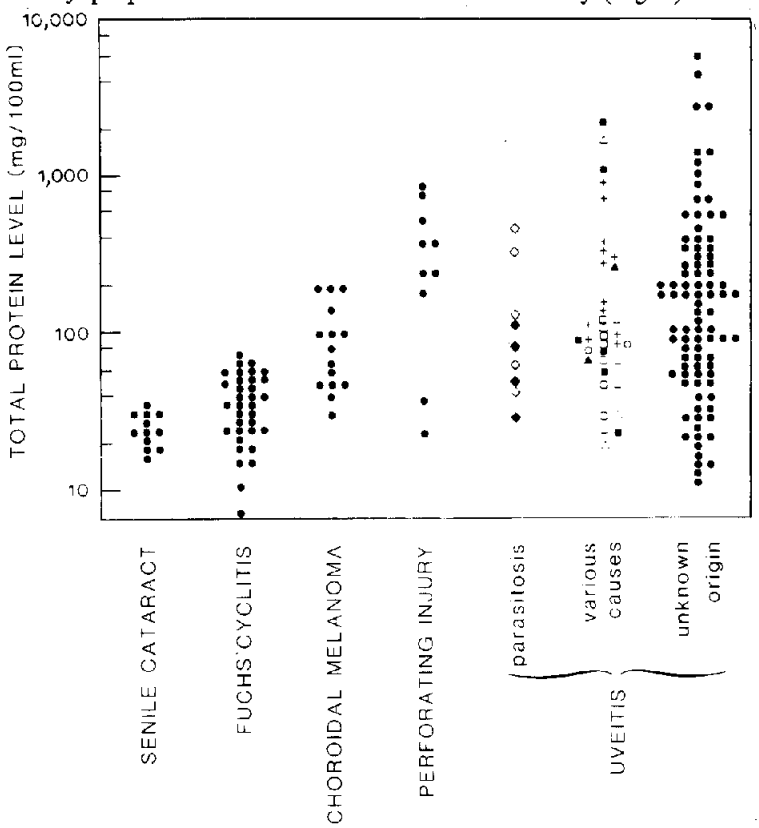

Figure 1. The total protein content of the aqueous humour in various ocular diseases.
◇ = onchocerciasis + = Behçet's syndrome
◆ = toxoplasmosis □ = sarcoidosis
■ = ankylosing spondylitis △ = Vogt-Koyanagi-Harada's syndrome
▲ = scleroderma ○ = Crohn's disease

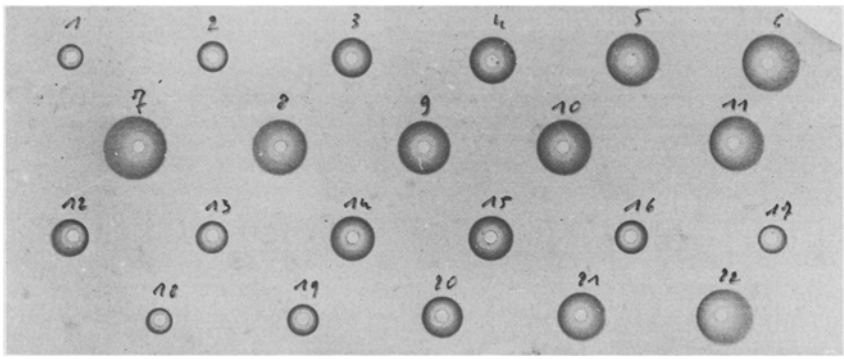

Figure 2. Quantitative determination of albumin in AH by single radial immunodiffusion (samples 8 to 15) compared with dilutions of a pool of sera from 500 blood donors of 1/720 to 1/60.

requires only 8 μl of sample for each quantitative determination. Its sensitivity is in the region of 1 to 10 μg/ml. The antisera were given to us by the Laboratory of Experimental Medicine and their specificity had been tested by immunoelectrophoresis and double diffusion according to Ouchterlony.

We determined the concentrations of 7 proteins in the AH and serum of 109 patients, and the results were divided into four groups according to the total AH protein level. Our reference group (A) was those patients whose AH had a total protein content lower than 32 mg/100 ml, which was considered the upper limit of normal. These patients were suffering from senile, immature cataract (n = 13) or inactive uveitis with no demonstrable ocular pathology (n = 6) at the time of the study. The other groups included patients suffering from active uveitis and their total AH protein contents were between 8 and 118 mg/100 ml for group B (n = 48), between 127 and 861 mg/100 ml for group C (n = 34) and between 1 061 and 5 951 mg/100 ml for group D (n = 8) (Fig. 3).

Since the protein content of the AH is generally considered to be an ultrafiltrate, the results were expressed as the ratio of their concentrations in the AH and in the serum (AH/S). This corrected for the effect of variations in serum proteins levels. As expected, the AH/S ratios for each protein increased in parallel with the total AH protein level. In confirmation of the notion of filtration, these ratios were inversely proportional to the molecular weights of the proteins studied, except in the group D, where no such size restriction was observed. There was, however, one exception to this : the AH/S ratio for transferrin was higher than expected from its molecular weight. This observation will be discussed in Chapter 2.

From these data, we have been able to determine some properties of the blood-aqueous barrier (BAB). We calculated a mean value for the size of hypothetical pores allowing proteins to pass along the BAB.

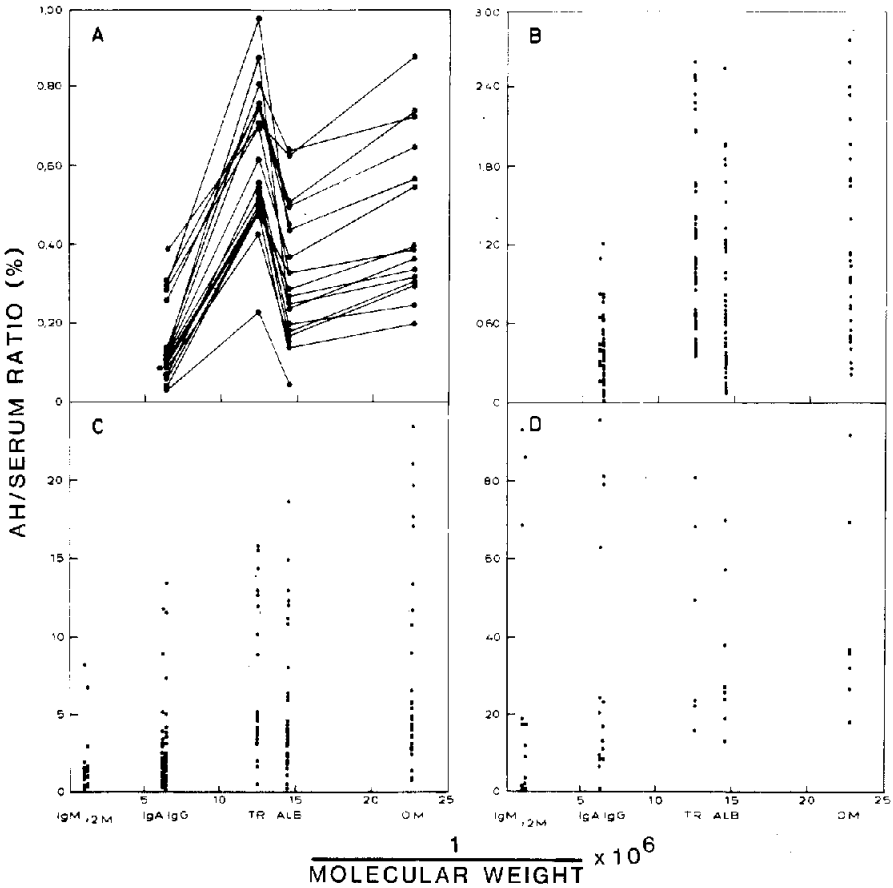

Figure 3. Relationship between the AH/serum concentration ratios of various proteins and their molecular weights in 109 patients. The samples were divided into 4 groups according to the total protein content of the AH:

group A (n = 19) : less than 32 mg/100 ml (normal)
group B (n = 48) : from 8 up to 118 mg/100 ml
group C (n = 34) : from 127 up to 861 mg/100 ml
group D (n = 8) : from 1 061 up to 5 951 mg/100 ml.

The solid lines, in group A, join the AH/serum values for individual patients. Protein molecular weights are expressed as their reciprocals $\times 10^6$.
OM = orosomucoid; ALB = albumin. TR = transferrin; α_2M = α_2-macroglobulin

Size of the hypothetical pores of the blood-aqueous barrier

To determine the mean size of hypothetical pores allowing the passage of serum proteins, we employed the mode of reasoning that Dive and Heremans (1974) used for the passage of serum proteins into the bile.

The exchanges through a porous membrane take place by filtration and by diffusion according to the size of the molecules and that of the pores. Solutes whose molecular size is negligible in comparison with that of the pores, cross these pores freely, as does the solvent. On the other hand, solutes whose size

is comparable with that of the pores, will pass less readily than the solvent. This restriction may be expressed, in terms of filtration, as a 'restricted pore surface' (Ar) in comparison with the 'total pore surface' (Ap), available for the passage of the solvent. In terms of diffusion, this restriction may be considered as a reduction of the diffusion coefficient (Dr) in comparison with the free diffusion coefficient (Do) in water. But at the molecular level, these two notions of restriction, that by filtration and that by diffusion, may be interchangeable, so that:

$$\frac{Ar}{Ap} = \frac{Dr}{Dp} \tag{1}$$

If a substance passes from compartment (a) into compartment (b) through a semi-permeable membrane, the Cb/Ca concentration ratio will be a function of the thickness (Δx) of the membrane, the pore surface (Ap), the quantity of solution passing the membrane (Qf) and the restriction on diffusion (Dr) and on filtration (Ar/Ap) of the substance studied. This relationship, which was established by Pappenheimer (1953) in his study of the permeability of capillaries, may be expressed by the following equation:

$$\frac{Cb}{Ca} = \frac{\frac{Dr\ Ap}{Qf\ \Delta x} + \frac{Ar}{Ap}}{\frac{Dr\ Ap}{Qf\ \Delta x} + 1} \tag{2}$$

For molecules of different sizes in solution in the same solvent crossing a membrane, the value of AP/Qf Δx remains the same (= K). Moreover, since Dr = (Ar/Ap) Do, equation (2) may be written as:

$$\frac{Cb}{Ca} = \frac{KDo\frac{Ar}{Ap} + \frac{Ar}{Ap}}{KDo\frac{Ar}{Ap} + 1} \tag{3}$$

The restriction on filtration, Ar/Ap, which is a function of the relative size of particles and pores, may be expressed according to the equation of Landis and Pappenheimer (1963) :

$$\frac{Ar}{Ap} = \left[2\left(1-\frac{\alpha}{r}\right)^2 - \left(1-\frac{\alpha}{r}\right)^4\right] \times \left[1 - 2.104\left(\frac{\alpha}{r}\right) + 2.09\left(\frac{\alpha}{r}\right)^3 - 0.95\left(\frac{\alpha}{r}\right)^5\right] \tag{4}$$

where α is the Stokes/Einstein diffusion radius for the substance studied, and r is the pore radius of the membrane. The Stokes/Einstein radius for a given substance is derived from its free diffusion coefficient in water.

To summarize, the pore radius of a semi-permeable membrane may be estimated from a set of Cb/Ca values corresponding to different particles of known Do and α. This may be calculated as following: (a) the A_r/A_p ratio for each substance is determined for a set of assumed values of pore radii (equation 4); (b) the coefficient K, which cannot be measured directly, is estimated by the method of least-squares from the experimental data for Cb/Ca and from the different Ar/Ap values by means of the equation (3); (c) from the K and Ar/Ap values corresponding to each of the assumed pore radii, one may calculate the theoretical Cb/Ca ratio for each substance and, by comparison with the actual values, estimate the best fitting value for the pore radius.

To calculate the mean pore size of the BAB, for a given group of patients, it was necessary to take into consideration the individual scatter of the AH/S values and the relative abundance of transferrin in the AH. This is the reason why, for the patients in groups A, B and C (Fig. 3), the AH/S value for each protein was referred to the patient's AH/S value for albumin. Albumin was chosen as reference protein because its relatively high concentration in the AH and its origin exclusively from serum give good evidence about the passage of serum proteins through the BAB. In this study, the patients of group D were not considered, because their individual AH/S values were the same for all the proteins studied. That signifies that in these patients with an AH protein content higher than 1 g/100 ml, the proteinogram of the AH is similar to that of the serum.

The correlation between the AH/S ratio for a given protein (prt) and that for albumin (alb) appeared to be linear (Fig. 4). In the regression equation:

$$\left(\frac{\text{AH}}{\text{serum}}\right)\text{prt} = \text{Yo} + \text{b}\left(\frac{\text{AH}}{\text{serum}}\right)\text{alb} \qquad (5)$$

the existence of a significant value for the intercept (Yo) implies that a certain amount of the protein concerned has been added to the AH independently of the passage of albumin across the BAB. This is the case for transferrin in groups A and B and for IgG in group B. For transferrin, this amount was between 0.34% and 0.49% of its serum concentration. For IgG, it was 0.16%. Active transport of IgG across the BAB is conceivable, as has been found in the placenta (Brambell et al., 1960; Gitlin, et al., 1964), but a local synthesis seems more likely. The injection of heterologous proteins into the rabbit's vitreous body results in the infiltration of the uvea by lymphocytes and immunoglobulin-secreting plasmocytes which may be recognized by immunofluorescence (Witmer, 1955a; Böke, 1965; Dieckhues, 1965–1967). In clinical practice, lymphocytes and plasmocytes may be observed in the AH of patients suffering from uveitis. Thus, it is probable that the abundance of IgG relative to albumin is due to intraocular immune reactions directed against a bacterial, parasitic or viral agent or to an autoimmune reaction against homologous tissue antigens.

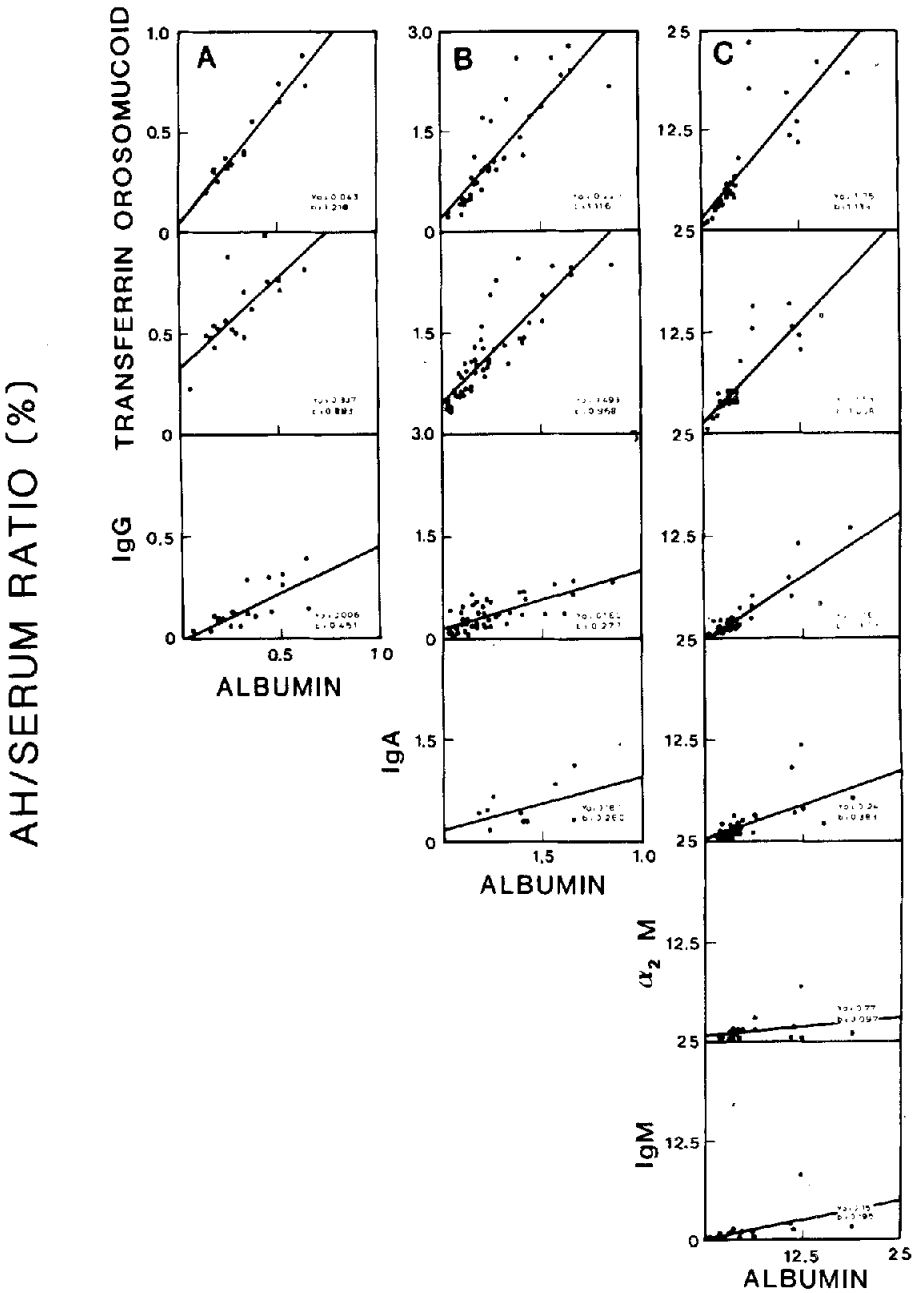

Figure 4. Correlation between the AH/serum concentration ratios of different proteins and that of albumin in 101 patients. The three groups A, B and C are the same as those used in Figure 3.

To clarify the meaning of the regression coefficient (b) in the equation (5), we have introduced the notion of relative clearance. In renal physiology, the clearance of a given substance is given by the ratio of its concentrations in urine and serum, multiplied by the urinary output. By analogy, it may be said that:

$$\left(\frac{AH}{serum}\right) prt \times V \tag{6}$$

corresponds to the aqueous clearance for a given protein where V is the volume of AH formed per unit time. By referring the aqueous clearance of a given protein to that of albumin, the factor V is eliminated, and the relative clearance of the protein studied may be obtained:

$$\text{relative clearance} = \left(\frac{AH}{serum}\right) prt : \left(\frac{AH}{serum}\right) alb \tag{7}$$

In fact, this relative clearance corresponds to the relative AH/S concentration ratio provided that the passage of the protein studied, through the BAB, is the same as that for albumin. Where there is local synthesis of a protein, the fraction synthesized in the eye must first be removed in order to establish the correlation between the transfer of this protein and its size. This is possible by studying, from the data of many patients, the correlation between the AH/S ratio for the protein concerned and that for albumin. The regression coefficient (b) in the equation (5) obtained in this manner will correspond then to the relative clearance of the protein studied.

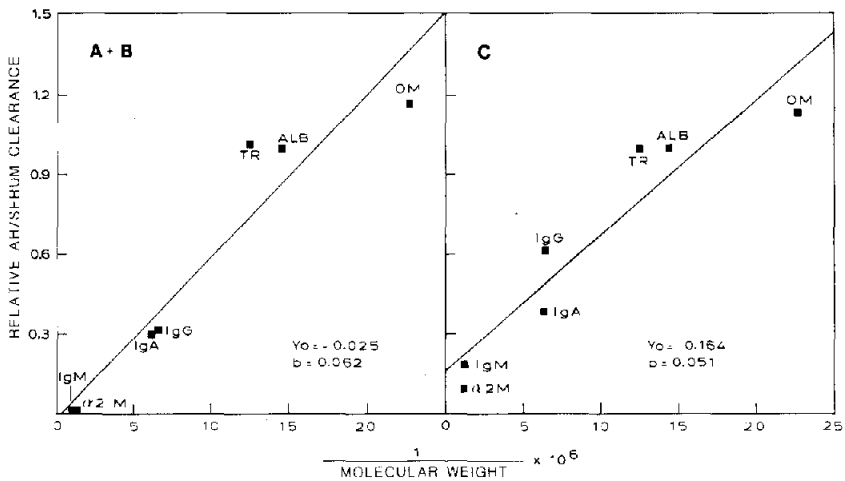

Figure 5. Correlation between the relative AH/serum clearance of various proteins and the reverse of their molecular weight in 101 patients. The three groups A, B and C are as in Figure 3. The data for groups A and B were pooled after it was shown that the differences between the two groups were not significant. OM = orosomucoid; ALB = albumin; TR = transferrin; α_2M = α_2-macroglobulin.

This study of the relative clearances has been applied to the different groups of patients. As data for only three proteins were available for this study in the case of group A, the data for these patients were pooled with those of group B after it was shown that the differences between the regression coefficients of the two groups were not significant. This gave four values of relative clearance for the groups (A + B).

It was also necessary to know if, in the groups (A + B) and C, a fraction of the proteins studied was not transferred from the serum into the AH by a process of bulk transport, i.e. a transport where the proportion of the different proteins is the same as in the serum. Thus, we studied the relationship between the relative clearances of the proteins and the reciprocal of their molecular weights (Fig. 5). This correlation appeared to be linear. In the regression equations, the intercept (Yo) was not significant ($P \geqslant 0.15$) implying the absence of bulk transport for the proteins.

As a result, by normalizing the clearance of the different proteins with respect to that of albumin, equation (3) becomes:

$$\text{Relative clearance} = \frac{\dfrac{K\left(Do\,\dfrac{Ar}{Ap}\right)prt + \left(\dfrac{Ar}{Ap}\right)prt}{K\left(Do\,\dfrac{Ar}{Ap}\right)prt + 1}}{\dfrac{K\left(Do\,\dfrac{Ar}{Ap}\right)alb + \left(\dfrac{Ar}{Ap}\right)alb}{K\left(Do\,\dfrac{Ar}{Ap}\right)alb + 1}} \qquad (8)$$

where the Do and Ar/Ap values for the protein studied and for albumin are indicated respectively by 'prt' and 'alb'.

By utilizing the relative clearance values obtained for the different proteins, the best fitting values for the coefficient K and for the pore radius, r, may then be calculated by the method of least-squares. By entering these estimates into equation (8), a theoretical correlation between the relative clearances and the molecular diffusion radii may be calculated (Fig. 6). On the whole, our data proved to be compatible with the assumption of the BAB behaving as an isoporous membrane with a pore radius of about 104 Å in the patients of groups (A + B) and about 113 Å for those in group C. These values are in good agreement with estimates for the major population of the pores of capillaries in the cervical region of the dog (110 Å) (Mayerson et al., 1960) and for the mean pore size of hepatic capillaries in the dog (102 Å) and human (127 Å) (Dive, 1970; Dive et al., 1974; Dive and Heremans, 1974).

Location of the molecular sieve

Before discussing the location of the molecular sieve, we shall consider the histology of the iris and ciliary processes which are responsible for the formation

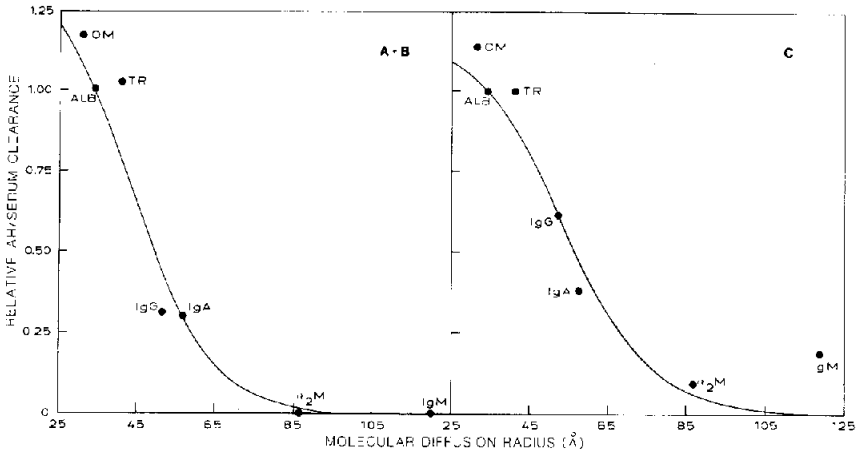

Figure 6. Relationship between the relative AH/Serum clearance of various proteins and their molecular diffusion radius (Å). This relationship was calculated from the equations of Pappenheimer, as modified by Dive et al. Results are from 101 patients, divided into the same 3 groups (A-B-C) on the basis of total AH protein level as in Figure 3. The data of the patients in groups A and B were pooled after it was shown that the differences between the two groups were not significant. The solid line represents the theoretical curve relating the relative clearance to the molecular diffusion radius of proteins assumed to be filtered through an isoporous membrane with a pore radius of 104 Å for groups (A + B) and 113 Å for group C. OM = orosomucoid; ALB = albumin; TR = transferrin; α_2M = α_2-macroglobulin.

of AH. The capillaries of the iris have a thick and poreless wall (Tousimis and Fine, 1959; Ikui et al., 1960) (Fig. 7), which is impermeable to proteins. This has been recently confirmed by Raviola (1977). The stroma is separated from the posterior chamber by a pigmented epithelium whose cells are connected by tight junctions. On the other hand, the stroma is in close contact with the AH of the anterior chamber through fissures between the pigmented and non-pigmented cells of the anterior layer of the iris. The stroma contains various cells. In addition to fibrocytes and characteristic cells such as the chromatophores and the 'Klumpenzellen' of Koganey with their pigment aggregates, cells of the immune system, such as macrophages, lymphocytes, plasmocytes and mastocytes, may also be found.

The ciliary processes, which are about 70 in number in man, constitute the 'ciliary ring', located behind the iris. The endothelium of their capillaries is provided with transcellular pores whose radius is between 100 and 600 Å (Holmberg, 1959; Pappas and Tennyson, 1962; Taniguchi, 1962; Missotten, 1964) and which are covered by a thin basal membrane. It is separated by a lamina of connective tissue from the ciliary epithelium. This epithelium consists of an outer layer of pigmented cells and an inner layer of non-pigmented cells, which are connected by tight junctions (Fig. 8).

Where among these diverse structures could the molecular 'sieve' for serum proteins be located? It could be in the walls of the ciliary capillaries or in the

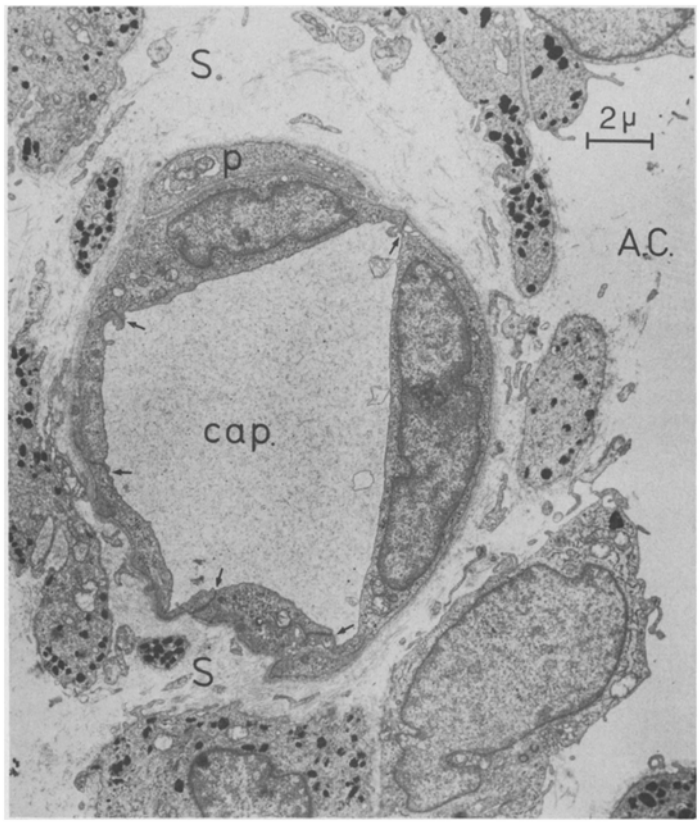

Figure 7. Electron micrograph of human iris (by courtesy of Prof. L. Missotten). cap. = capillary; p = pericyte; s = iridial stroma; A.C. = anterior chamber of the eye. Arrows show tight junctions connecting the endothelial cells of the capillary walls.

ciliary epithelium. Indeed peroxidase, after intravenous injection into the monkey, seemed to cross the wall of the ciliary capillaries relatively rapidly, and to be blocked at the level of the tight junctions of the inner (non-pigmented) cell layer (Vegge, 1971; Shabo and Maxwell, 1972). If this epithelium was responsible for the sieve effect, it would be necessary to accept a transcytoplasmic pathway, because cytologists think that the tight junctions are hermetically sealed (Staehelin, 1974; Berkallof et al., 1977). A transcytoplasmic transfer is accepted for IgG across placenta (Brambell et al., 1960; Gitlin et al., 1964), and for IgA and IgM across enterocytes (Brown et al., 1977) and hepatocytes (Jackson et al., 1978; Orlans et al., 1978; Fisher et al., 1979). In the experiments already cited, cytoplasmic peroxidase-filled vesicles were observed in the inner (non-pigmented) cell layer of the epithelium. In these experiments, however, the eyes were removed for study between 5 and 60 minutes after intravenous injection of peroxidase, too short a time to allow

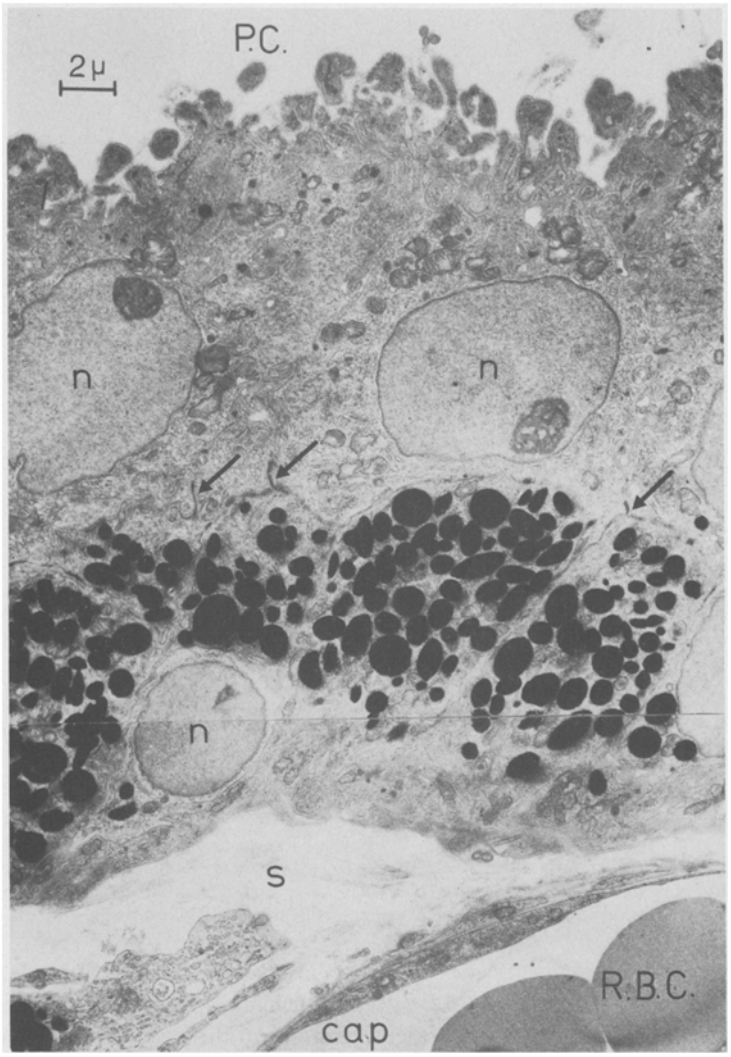

Figure 8. Electron micrograph of human ciliary process (by courtesy of Prof. L. Missotten). R.B.C. = red blood cell; cap = capillary; s = stroma; n = nuclei of the pigmented and non-pigmented cells of the ciliary epithelium; P.C. = posterior chamber of the eye. Arrows show tight junctions connecting the non-pigmented cells of the ciliary epithelium.

the importance of a possible transcytoplasmic transfer to be estimated. If, in reality, the ciliary epithelium constitutes the 'sieve', it would be necessary to demonstrate that proteins do not by-pass this epithelium. According to the hypothesis of a by-pass, the proteins of the ciliary stroma would migrate, probably without restriction, through the root and the stroma of the iris, before being discharged into the AH of the anterior chamber (Raviola, 1977).

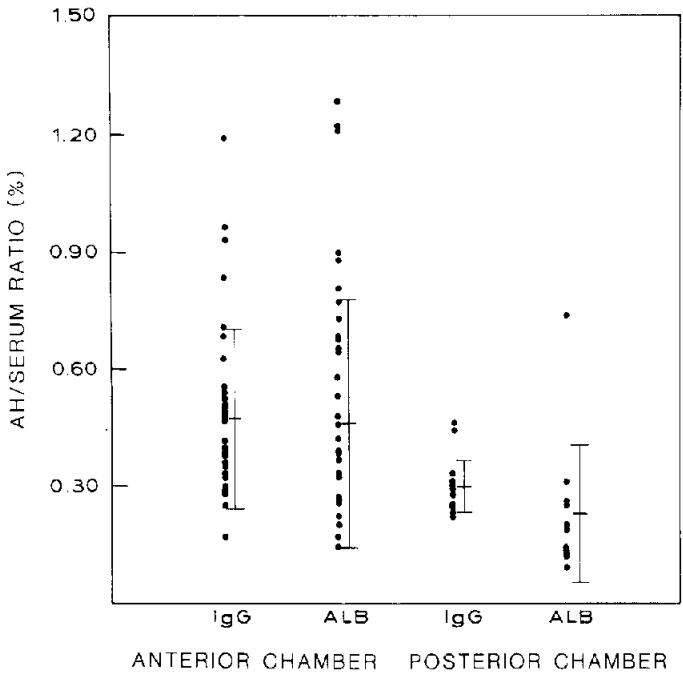

Figure 9. AH/Serum concentration ratios for albumin (ALB) and IgG in rabbits. AH samples were taken from the anterior chamber of the eye in 40 rabbits and from the posterior chamber in another 14. Bars show the means of the AH/serum values ± 1 standard deviation.

Against this hypothesis is the fact that we have not found significant differences of the concentrations of albumin and IgG between the anterior and the posterior chamber of the rabbit ($P > 0.20$) (Fig. 9). Moreover, it should be noted that in the rabbit, the AH/S values for albumin and those for IgG were similar, which is not the case in man. This implies that the BAB is probably less selective in the rabbit.

Since the data in the literature on the passage of proteins across the ciliary epithelium are inconclusive, it is quite possible that the 'sieve' is located in the wall of the ciliary capillaries. From the differences in concentration of substances of various sizes across the wall of various blood capillaries, it is valid to compare the capillary wall, in general, to a semi-permeable membrane. The most numerous pores would have a radius in the size range of 40 Å (Pappenheimer, 1953) to 110 Å (Mayerson et al., 1960), while a minority population would range in size from 250 Å to 350 Å (Grotte, 1956). The junctions between capillary endothelial cells would appear, by electron microscopy, to be impermeable to molecules with a radius greater than 10 Å, while those between endothelial cells of the postcapillary venules are impermeable to molecules with a radius greater than 30 Å (Palade et al., 1978). Since these values are lower than those we have calculated for the size of the 'theoretical

pores', it is unlikely that the 'sieve' is located at the level of the intercellular spaces. Is it not possible then, that proteins cross the endothelial cells themselves? When these cells were examined by electron microscopy, they appeared to contain in their cytoplasm many vesicles with a radius of 150 to 450 Å (Taniguchi, 1962; Bruns and Palade, 1968). Some vesicules communicated with the capillary lumen or the pericapillary spaces through an aperture of radius 50 to 200 Å, covered by a small membrane, the diaphragm. These vesicles appeared to be responsible for a small part of the transcytoplasmic transfer of macromolecules, such as ferritin (MW = 450 000) and peroxidase (MW = 43 000) (Bruns and Palade, 1968; Palade et al., 1978) by both endocytosis and exocytosis. Other vesicles forming chains of two or more units were responsible for the formation of transendothelial tunnels opening on both sides of the wall (Simionescu et al., 1975). These tunnels, which were also observed in the endothelial cells of the ciliary capillaries, could correspond to the 'theoretical pores' of our calculations. More specifically, the 'sieve' for the transfer of proteins could be located at the level of the diaphragm and of the strictures (radius 50 to 200 Å) observed at the junction of the vesicles and at the ends of the tunnel. Thus, our theoretical values of 104 and 113 Å are compatible with the hypothesis of a transcellular transfer.

In cases of inflammation we have seen that the AH protein levels were increased. Then, it may be asked whether the presence of additional serum proteins in the AH is the consequence either of a bulk transfer through gaps in the capillary walls or of an increased transendothelial transfer. Since no bulk transfer was observed in the groups of patients (A + B) and C (shown by the lack of a significant intercept Yo in the diagrams of the Fig. 5), it is probable that the increased permeability was mostly an exaggeration of the physiological process. There was a filtration, but through 'theoretical pores' which were either slightly larger or more numerous. Since the mean pore sizes were practically the same for the groups (A + B) and C, this must indicate an increase in the number of pores, which could result from an increased number of intracytoplasmic vesicles or from the formation of pores at other places, for example, between the endothelial cells. After intravenous injection of endotoxin into the rabbit, the transfer of colloid particles through the inflamed wall of the ciliary capillaries appeared, by electron microscopy, to be intercellular (Pappas and Tennyson, 1962). Moreover, after local or general administration, into animals, of substances characteristic of the inflammatory response such as histamine or some prostaglandins, the intercellular spaces of the ciliary (Whitelocke et al., 1973; Cole, 1974) and iridial (Ashton and Cunha-Vaz, 1965; Shakib and Cunha-Vaz, 1966) capillaries were seen to be enlarged, probably because of contraction of the endothelial cells (Unger et al., 1975), leading to a transfer of macromolecules. Furthermore, it is likely that the alterations of the permeability in the wall of the ciliary capillaries are responsible for oedema of the ciliary processes with, as consequence, a disjunction of the inner (non-pigmented) cell layer of the epithelium (Meyers et

al., 1975; Vegge et al., 1975). Such a disjunction can be also observed after a puncture of the anterior chamber (Neufeld and Sears, 1973; Unger et al., 1975).

Chapter 2

Intraocular transferrin

Transferrin (Tr) is a glycoprotein which can bind two ferric ions per molecule. Its molecular weight is about 80 000 and its serum concentration is normally about 200 mg/100 ml (Schultze and Heremans, 1965). Two principal roles can be assigned to Tr: the transport of iron, and a bacteriostatic activity due to its great affinity for iron. By depriving the medium of free ferric ions, Tr can inhibit the growth of bacteria (Rogers, 1967). These can secrete chelating agents whose the affinity for iron is sometimes higher, for some pathogenic strains, to that of Tr. Some of these chelating agents have been well characterized, such as enterochelin, which is a cyclic trimer of 2:3 dihydroxybenzoyl serine, isolated from *Salmonella typhimurium* (Pollack and Neilands, 1970), *Escherichia coli* and *Klebsiella aerogenes* (O'Brien and Gibson, 1970). Antibodies blocking the liberation or the reabsorption of these chelating agents could reinforce the bacteriostatic effect of Tr (Rogers, 1975).

Because of the impossibility, in man, of performing experiments, and the difficulty of obtaining large numbers of 'normal' AH samples, quantitative analyses for Tr were performed in the AH and serum of rabbits and guinea pigs. Moreover, because of the well known existence of exchanges by diffusion between the aqueous and vitreous humours, we analysed rabbit vitreous humour. We were able to show that the electrophoretic mobility of the Tr in the vitreous humour was different of that in the AH and serum. Finally, we investigated the intraocular abundance of this protein. This was done by studying the transfer, in the eye, of heterologous or homologous ^{125}I-transferrin.

Quantitative analyses in animals

Quantitative determinations in the serum and intraocular fluids of animals showed that the AH/S concentration ratio of Tr was slightly higher than that of albumin in rabbits ($n = 39$) and guinea pigs ($n = 14$), while the vitreous/S ratio for Tr was significanrly higher in rabbits ($n = 18$). The correlations between the AH/S and vitreous/S ratios for Tr and those for albumin are presented in Figure 10.

For the AH, this correlation appeared linear with an intercept (Yo) which was statistically significant. This implies that a certain amount of Tr has been added to the AH independently of the passage of albumin across the BAB.

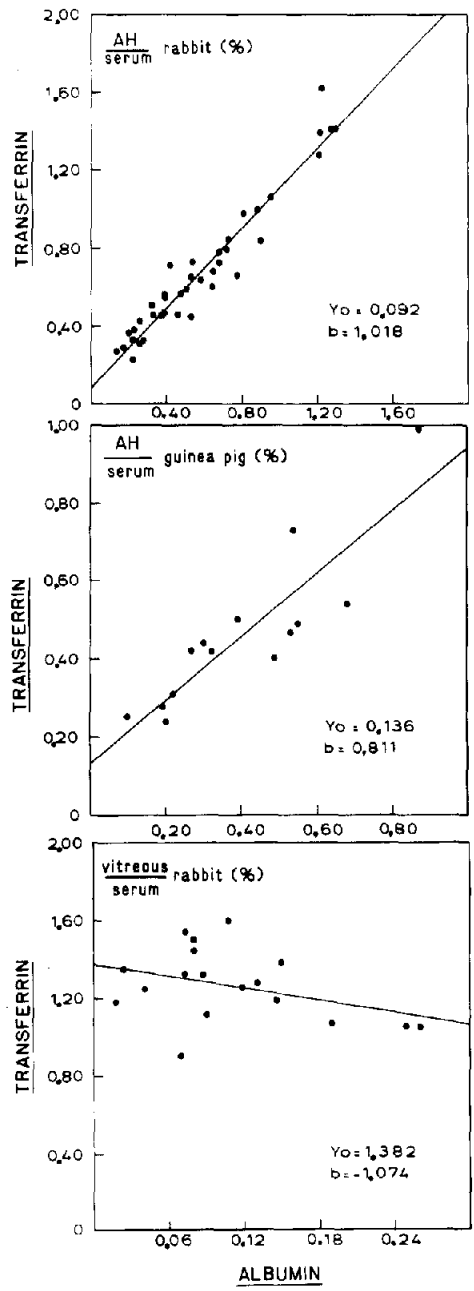

Figure 10. Correlation between the intraocular fluids/serum concentration ratios for transferrin and for albumin in rabbits and guinea pigs. The negative slope of the results for rabbit vitreous humour is not significant (P = 0.11).

The amount of 'independant' Tr in the rabbit and guinea pig AH reached a maximum of 0.09% ($P < 0.005$) and 0.14% ($P < 0.04$) respectively of the serum concentration of Tr.

For the vitreous, it must be emphasized that there was no correlation between the ratios for Tr and those for albumin. In other words, the presence of Tr in the vitreous humour was totally independant of that of albumin. The amount of Tr in the vitreous humour reached 1.38% of its concentration in the serum.

Properties of intraocular transferrin in rabbits

Serum Tr consists of a single polypeptide chain (Green and Feeney, 1968; Mann et al., 1970) to which are attached two oligosaccharide chains terminating in sialic acid residues. There are two of these residues in the rabbit (Baker et al., 1968; Palmour and Sutton, 1971; Scharmann et al., 1971) and four in man (Jamieson, 1965; Scharmann et al., 1971). Each molecule of Tr contains two specific sites iron-binding with high association constants (Aisen, 1970). By thermodynamic and spectroscopic studies, these two sites were shown to be very similar (Aasa et al., 1963; Aisen et al., 1966). The structure of the molecule of Tr is strongly symmetrical (Williams, 1974).

Crossed immunoelectrophoresis performed with antisera directed against rabbit albumin and Tr demonstrated the relative over-abundance of Tr in the vitreous humour (Fig. 11).

In agarose gel electrophoresis at pH 8.6, Tr from the AH appeared to migrate just in the same way as that from serum. Tr from the vitreous humour, on the other hand, appeared to be distributed into three bands, the fastest of which corresponded to serum Tr (Fig. 12) (Dernouchamps et al., 1975). After sialidase treatment, according to a method described elsewhere (Dernou-

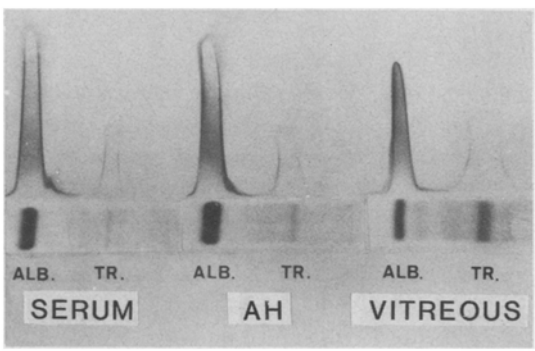

Figure 11. Crossed immunoelectrophoresis of rabbit serum, aqueous humour and vitreous humour. Each sample was taken from a pool from a number of animals, the serum after dilution to 15 mg total protein/ml, and the humours after concentration to 15 mg/ml. Crossed immunoelectrophoresis was by the method of Laurell (1965). ALB. = albumin; TR. = transferrin. (Photograph reproduced from Dernouchamps et al., Ophthalmologica 170: 72–83, 1975 with permission of S. Karger AG, Basel)

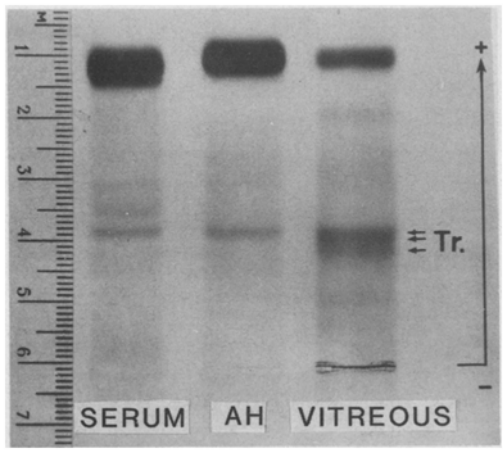

Figure 12. Agarose gel electrophoresis at pH 8.6 of pools of sera, aqueous humour (AH) and vitreous humour samples from rabbits. The pool of sera was diluted and the pools of aqueous and vitreous humours concentrated to a total protein level of 15 mg/ml. TR. = transferrin. (Photograph reproduced from Dernouchamps et al., Opthalmologica 170: 72–83, 1975 with permission of S. Karger AG, Basel).

champs et al., 1975), all variants of Tr, from aqueous and vitreous humours or serum, were converted into one slow-moving component co-migrating with the slowest component of the Tr in the vitreous humour (Fig. 13).

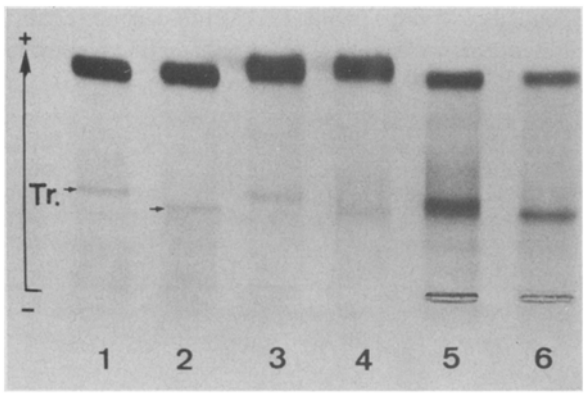

Figure 13. Agarose gel electrophoresis at pH 8.6 of: 1. = pool of rabbit sera diluted to a total protein level of 15 mg/ml; 2. = As 1, after treatment with sialidase; 3. = pool of rabbit aqueous humour samples concentrated to a total protein level of. 15 mg/ml; 4. = As 3, after treatment with sialidase; 5. = pool of rabbit vitreous humour samples concentrated to a total protein level of 15 mg/ml; 6. = As 5, after treatment with sialidase. TR. = transferrin. (Photograph reproduced from Dernouchamps et al., Ophthalmologica 170: 72–83, 1975, with permission of S. Karger AG, Basel).

The simplest hypothesis to account for these observations is that, in the rabbit, serum and AH Tr, as well as the fastest component of that in the vitreous humour, has two sialic acid residues per molecule. The remainder of the vitreous humour Tr contains only one or no sialic residue. Only chemical analysis can confirm or refute this hypothesis. However, the absence of one or several sialic acid residues has already been suggested for the Tr of rabbit (Baker et al., 1968) and rat (Morgan, 1968) milk, in foetal serum (Parker and Bearn, 1962), and in human cerebrospinal fluid (Laterre, 1965) and vitreous humour (Manuel, 1969). In addition, chemical analyses have shown that the slower electrophoretic mobility of conalbumin or ovotransferrin (an egg-white protein) was due to a reduction of sialic acid content (Williams, 1968).

If, in reality, a part of the Tr of the rabbit vitreous humour is devoid of sialic acid, this could arise from the action of sialidase in the eye, or from the intraocular synthesis of partially sialylated Tr. In favour of the first hypothesis is the presence of sialidase in the rat aqueous and vitreous humours (Tulsiani et al., 1973). We shall discuss later about the possibility of a local synthesis.

By double diffusion in agarose gel, it was shown that no antigenic difference exists between the serum Tr and that of aqueous and vitreous humours (Fig. 14), the antiserum used being directed against rabbit serum Tr. Thus these results indicate that Tr, in the aqueous and vitreous humours, possesses all the antigenic determinants which are present in serum Tr. Nevertheless, the presence of specific determinants for intraocular Tr cannot be excluded, because we had no antiserum directed against the Tr of aqueous and vitreous humours.

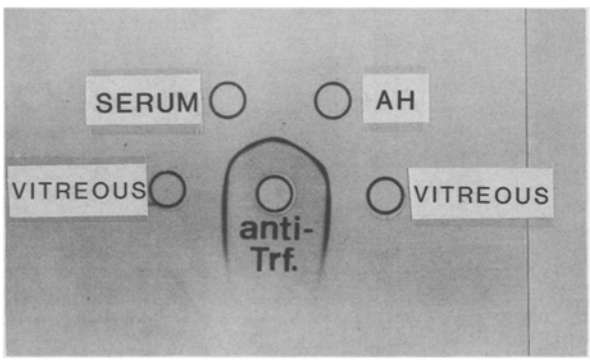

Figure 14. Double immuno-diffusion (Ouchterlongy) in argarose gel at pH 8.6. Serum = pool of rabbit sera diluted to a total protein level of 15 mg/ml.; AH = pool of rabbit aqueous humour samples concentrated to a total protein level of 15 mg/ml.; Vitreous = pool of rabbit vitreous humour samples concentrated to a total protein level of 15 mg/ml.; Anti-Trf. = goat antiserum to rabbit serum tranferrin. (Photograph reproduced from Dernouchamps et al., Ophthalmologica 170: 72–83, 1975 with permission of S. Karger AG, Basel).

Transfer of serum transferrin into the rabbit intraocular fluids

To study, in the rabbit, the transfer of Tr from serum into the intraocular fluids, we used human Tr, which is recognizable by its special antigenic determinants, or rabbit ^{125}I-transferrin.

In a first experiment, 800 mg of purified human Tr (Behringwerke, Marburg/Lahn, Germany) were given intravenously to an adult rabbit. Forty eight hours later, samples of AH, vitreous humour and serum were taken for quantitative analyses of the foreign protein as well as the autologous albumin and Tr. In contrast to the behaviour of rabbit Tr, human Tr was not found to be in excess relative to albumin in AH and vitreous humour (Fig. 15). If the experiment was of sufficiently long duration to allow equilibrium between blood and eye compartments, we must presume that the two types of Tr behave differently. We may imagine that heterologous Tr reaches the fluids of the eye by simple filtration, while active transport, selective concentration because of an absence of elimination, or local biosynthesis may all occur with autologous Tr.

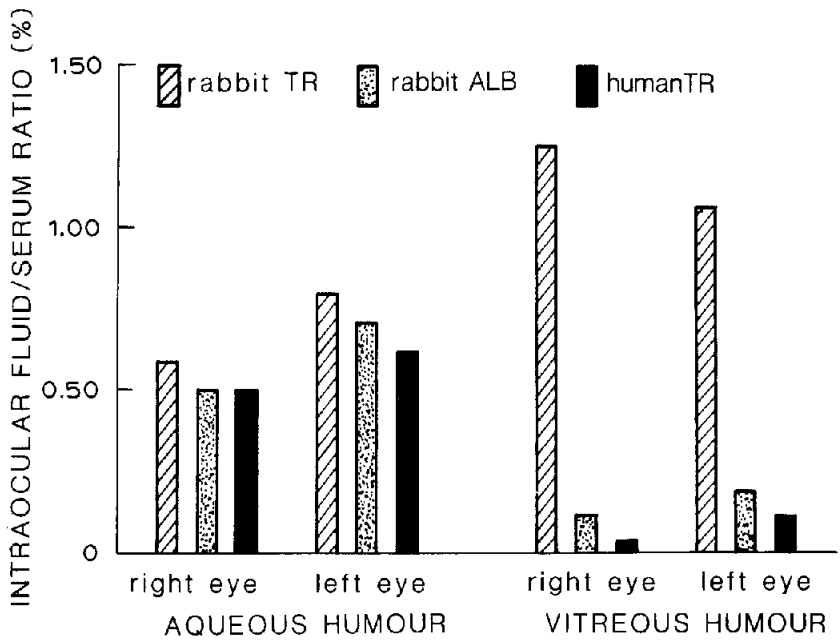

Figure 15. Quantitative determination of rabbit albumin and transferrin and of human transferrin in the intraocular fluids of a rabbit, 48 hours after intravenous injection of 800 mg human transferrin, TR = transferrin; ALB = albumine.

If there is active transport of autologous Tr, then the lower AH/S concentration ratio for human as opposed to autologous Tr could be explained by

the absence of receptors for the foreign protein in the ciliary eptithelium of the rabbit. To decide between these alternatives, a second experiment was performed, whereby 1 mg of homologous ^{125}I-transferrin was given intravenously to two rabbits. Experimental details are published elsewhere (Dernouchamps et al., 1975). The electrophoretic mobility of this labelled Tr was found to be the same as that of non-labelled Tr, indicating the absence of substantial changes to the protein during the labelling. Aqueous and vitreous samples were taken from the right eye after three days and from the left eye after four days. A serum sample was taken at the same times. To avoid contamination of the samples by free iodine, which might have falsified the results, the radioactivity in the proteins alone was counted after their precipitation by trichloracetic acid. The specific activity of Tr was found to be equivalent in serum and AH, but clearly lower in vitreous humour (Fig. 16). This implies that in vitreous humour the labelled protein was obviously diluted by non-labelled Tr.

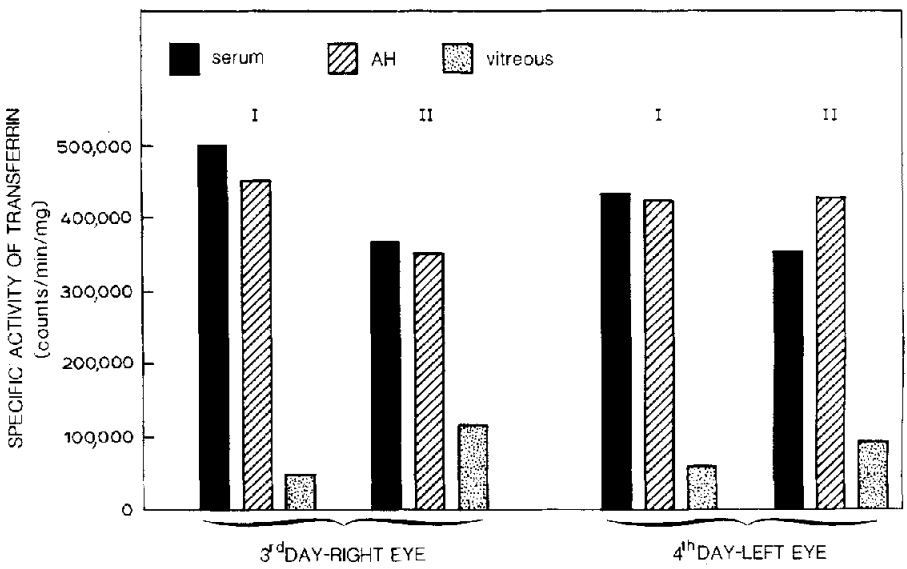

Figure 16. Specific activity (in counts/min/mg) of transferrin in the serum and intraocular fluids from 2 rabbits (I-II), after intravenous injection of 1 mg homologous ^{125}I-transferrin (1.45 mc). Aqueous and vitreous humour samples were taken from the right eye of each animal on the 3d day after intravenous injection and from the left eye on the 4th day. Serum samples were taken at the same times. AH = aqueous humour.

If an equilibrium between blood and eye compartments was obtained after three or four days, the results of this experiment are against an active transport of serum Tr into the vitreous humour. In addition, it is probable that after three or four days, an accumulation of Tr by a selective decrease of

elimination would also be found with ^{125}-I-Tr. In consequence, there would also have been a decrease of the dilution observed for this labelled protein. Thus, the hypothesis of a local synthesis seems the most probable. Tr is mainly synthesized in the liver, but the existence of other sites of production has been proven. The synthesis of Tr has been demonstrated, by incorporation of ^{14}C-amino acids, in the spleen, bone marrow (Morgan, 1969), submaxillary gland, mammary gland, testis, ovary (Thorbecke et al., 1973) and lymphocytes (Soltys and Brody, 1970). Thus, intraocular production of Tr is not impossible. Because the concentration of Tr is higher in vitreous humour than in AH, it would occur in the posterior pole of the eye; from the vitreous humour, Tr would diffuse into AH. Nevertheless, the hypothesis of an active transport specific for one special type of Tr (the slow-moving band in electrophoresis) cannot be excluded.

Whatever the mechanism of this accumulation of Tr in the intraocular fluids, it may be asked what is the biological significance of this phenomenon. The answer lies perhaps in the relatively high concentrations of iron-binding proteins (not only Tr, but also lactoferrin, a protein which is similar to Tr) in various biological fluids, such as cerebrospinal fluid and various exocrine secretions (milk, tears, saliva, nasal and bronchial secretions, seminal fluid). There is also an abundance of lactoferrin in the specific granules of neutrophile leucocytes. It is tempting to believe that this distribution of Tr and lactoferrin in the various biological fluids is because of their bacteriostatic properties.

Chapter 3

Immunopathology of the uvea

Many studies of immunoglobulins and antibodies in AH suggest that immune reactions play an important role in numerous diseases of the uvea. However, the relative importance of the different pathogenic mechanisms, i.e. response to infection, autoimmune reactions or deposits of immune complexes, is not well known. Our contribution to this study is based on the specific quantitative determination of immunoglobulins, and the detection of antigen-antibody complexes and rheumatoid factor in the AH and serum of patients suffering from various disorders of the uvea. We shall discuss the results obtained with regard not only to the origin of the disease, but also to the clinical interest of these analyses.

The specificity of antibodies has been used by various workers to try to determine the cause of uveitis. However, their presence in serum has only a suggestive value, while their detection in AH is clearly more significant, especially if an intraocular synthesis is suggested by the AH/S ratio for

antibody titer. This ratio must, of course, be corrected by that of the respective immunoglobin concentrations (Goldmann and Witmer, 1954):

$$Q = \frac{\text{AH antibodies}}{\text{serum antibodies}} \Big/ \frac{\text{AH immunoglobulins}}{\text{serum immunoglobulins}}$$

If the antibodies in AH originate only from the serum, the quotient Q will be equal to one. In case of values higher than unity, a decreased catabolism or a selective transfer of antibodies through the BAB could be considered, but an intraocular synthesis, as discussed previously (Chapter 1), seems to be the most probable mechanism.

The pathogenic mechanisms

Response to infections. In some cases of uveitis, it has been possible to determine the etiology by using the antibody quotient: toxoplasmosis (Remky, 1965a; Desmonts, 1966; Witmer, 1967; Michiels, 1968), tuberculosis (Witmer, 1955b, 1962; Remky, 1958, 1960; Schlaegel et al., 1961), strepthococcal infections (Remky, 1958, 1960; Laffers and Bozsoky, 1959), leptospirosis (Witmer, 1954), syphilis and viral infections (Campinchi et al., 1967; Martenet, 1970a). However, in clinical practice, this type of testing is limited by the small volume of AH that can be sampled, thus restricting the testing of antibody activity to only a small number of antigens. It should also be noted that the time of ocular puncture is crucial. Indeed, after intravitreal injection of heterologous proteins into rabbits, the antibody quotient has been found higher than unity after 10 days, reaching a maximum after 28 days and then decreasing (Fig. 17) (Michiels and Dernouchamps, 1976).

Autoimmune reactions. The most frequently studied ocular autoantigens are those of lens and uveal origin. According to the classical theory, as the lens proteins are isolated from the general circulation because of the early closing of the lens capsule, they cannot induce immune reactions including tolerance during foetal life. Traumatic rupture of the capsule, after birth, will lead to the release of lens proteins, now able to induce an immune response. This classical theory must now be reconsidered: contrary to accepted opinions, lens proteins are present at very low concentration in normal human as well as animal AH (Maisel, 1963). This implies that there is a definitive immune tolerance towards these proteins. In fact, this tolerance would seem to be only partial, as it has been shown that, in normal animals, some B-lymphocytes (cells of humoral immunity) were able to fix autoantigens such as thyroglobulins (Clagett and Weigle, 1974) and lens proteins (Marak et al., 1979), implying that only the T-lymphocytes are tolerant. Since T-lymphocytes are necessary for the production of antibodies by B-lymphocytes (helper action) against most antigens, no antilens antibodies will be produced in normal humans (Halbert et al., 1957; Luntz, 1964;

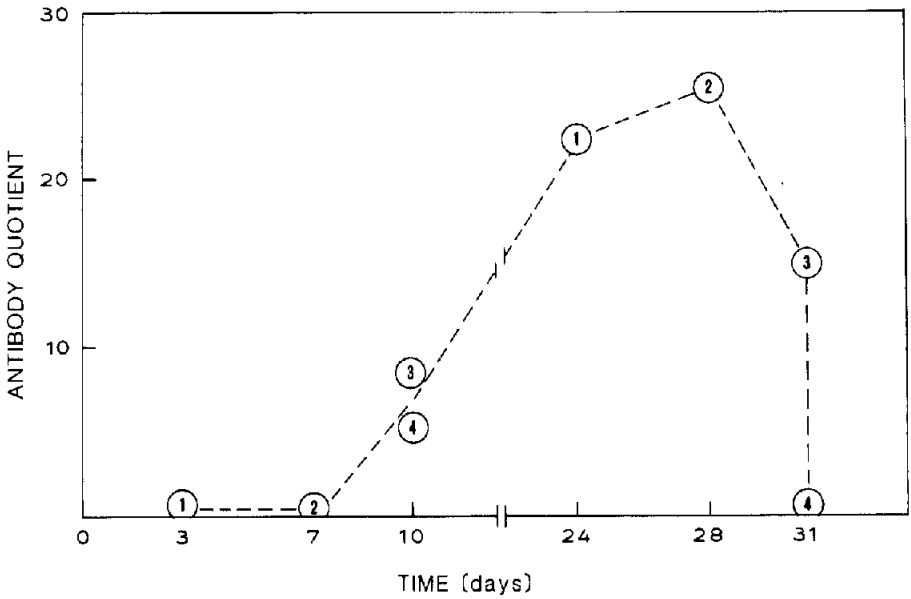

Figure 17. After intravitreal injection of 1 mg bovine gammaglobulin (BGG) into 4 adult rabbits, AH and serum samples were taken regularly for one month. The concentrations of IgG, IgA and IgM and also that of anti-BGG antibodies were determined by single radial immunodiffusion and the results expressed in mg/100 ml by comparison with standard solutions. Technical details of the preparation of these standard solutions were given elsewhere (Michiels and Dernouchamps, 1976). The antibody quotient was calculated as:

$$\frac{\text{(anti-BGG antibodies) AH}}{\text{(anti-BGG antibodies) serum}} \Big/ \frac{\text{(IgG + IgA + IgM) AH}}{\text{(IgG + IgA + IgM) serum}}$$

The numbers 1 , 2 . . . refer to the different rabbits tested.

Wirostko and Spalter, 1967). This could explain the rarity of inflammatory reactions observed in the eye after the discission of congenital cataract.

On the other hand, it must be pointed out that antilens antibodies are often detected in the serum of patients suffering from uveitis (between 44 and 60% of the cases). These antibodies were detected by complement fixation test (Perkins and Wood, 1964) or passive hemagglutination (Luntz, 1968), using bovine lens extracts. If these tests were really detecting autoantibodies, we still need to know if these antibodies play a pathogenic role. In bacterial or viral diseases, a great variety of autoantibodies may appear (Allison, 1977), probably owing to a mitogenic effect of the infectious agents on B-lymphocytes. This may be observed, for example, in cases of herpes virus disesase (Rosen et al., 1977) or as a result of the action of bacterial products such as endotoxins (Izui et al., 1977, 1979). After injection into animals, endotoxins can induce the production of various autoantibodies, for example, anti-DNA and anti-IgG autoantibodies (rheumatoid

factor). Another mechanism responsible for the breaking of immune tolerance could be the appearance of new antigenic determinants capable of stimulating T-(helper) lymphocytes, and then the production of autoantibodies because of the stimulating action of these T-cells on the B-cells. The new determinants may arise, for example, from the denaturation or degradation of lens proteins by an infectious agent (Allison, 1977). This could explain why the intravitreal injection of lens proteins in the rabbit will only provoke uveitis with intraocular formation of antilens antibodies when herpes virus is administered simultaneously (Witmer and Martenent, 1964).

Antiuvea autoantibodies have also been detected in many cases of uveal inflammations, and their level in serum was highest when the uveitis was bilateral and of long standing (Aronson et al., 1966; Martenet, 1970b). Since Elschnig (1910), it has been assumed that the uveal antigen responsible was melanin pigment; but melanin has not yet been demonstrated to have antigenic properties (Campinchi et al., 1970). The basal membrane of the uveal vessels has been also suspected; however, antimembrane antibodies have never been found in the serum of patients with uveitis (Sery and Huang, 1968). Finally, since the work of Wacker and Lipton (1965), and Faure et al. (1973), demonstrating the antigenic properties of the retinal photoreceptor outer segments in the induction of experimental uveitis, it may be asked whether the antiuvea autoantibodies are not in fact directed against retinal antigens. Indeed, the uveal preparations used for the demonstration of autoantibodies could have been contaminated by retinal antigens. Injection of autologous retinal extracts, emulsified with complete Freund's adjuvant, into the footpads of guinea pig produced a long-lasting uveoretinitis (Faure, 1980).

It must be emphasized that the detection of autoantibodies does not necessarily imply the existence of an autoimmune disease. Further, many autoimmune reactions may promote either the elimination of altered autoantigens or the regulation of the immune response. For example, rheumatoid factor, which is an autoantibody directed against the patient's own immunoglobulins (Kunkel et al., 1959), favours the phagocytosis of immune complexes by macrophages (Van Snick et al., 1978).

Genetic factors play an obvious role in various autoimmune diseases such as lupus erythematosus and ankylosing spondylitis. Since various forms of uveitis seem to be dependent on this immune mechanism, it may be asked whether genetic factors are not at the basis of these diseases. Particularly interesting as genetic markers in this respect are the major histocompatibility antigens (HLA).

The HLA system (H for human, L for leucocyte and A for first locus A) can be used to differentiate individuals by their possession of various membrane antigens. These tissular groups are dependent on four genetic loci, named A, B, C, and D. These are closely linked between themselves and are found on the 6th pair of chromosomes. A locus HLA-Dr has been recently

defined, but its identity with HLA-D is still discussed. It would correspond to the 'T' region of the H-2 complex on the 17th chromosome of the mouse and, by analogy with this 'T' region, would be the regulator of immune responses (Dausset, 1977). The major histocompatibility group is characterized by a great variety of antigens. For example, the locus B possesses 40 alleles (Committee for HLA nomenclature, 1980).

Various associations between HLA antigens and diseases have been observed. A clear example is that of ankylosing spondylitis: 90% of patients suffering from this disease possess HLA-B27 antigen, whereas this antigen is found in only 4 to 8% of the normal population (Schlosstein et al., 1973; Brewerton et al., 1973). HLA-B27 antigen is also present in 55% of patients suffering from acute anterior uveitis (Brewerton et al., 1973; Mapstone and Woodrow, 1975). Although some of these patients were also suffering from ankylosing spondylitis, the risk of acute anterior uveitis for normal people with HLA-B27 is 15 to 20 times higher than that for people without HLA-B27 (Char, 1978). A second example of HLA-disease association is constituted by the HLA-B5 antigen and Behçet's syndrome (Ohno et al., 1978). This syndrome is characterized by recurrent uveitis with hypopyon, by buccal and genital aphtae and sometimes by various other manifestations which may be meningoencephalitic, arthralgic, cutaneous, pulmonary, intestinal, renal and muscular. Another example is the Vogt-Koyanagi- Harada's syndrome which is frequent in Japan and associated with BW54 antigen (Tagawa and Suguira, 1978). It is a total uveitis with serous retinal detachment. This form of uveitis is associated with meningo-encephalitic signs, poliosis, alopecia, vitiligo and hypoacousia. The HLA-disease association gives rise to many hypotheses, but none is yet proven.

Immune complexes diseases. Described after the first serotherapies, serum sickness (von Pirquet, 1906) can be complicated, in some cases, by uveitis (Theodore and Lewson, 1939; Sedan and Guillot, 1955). Subsequently, it has been shown that repeated injections of a foreign protein into animals produced a glomerulonephritis similar to that of human serum sickness (Benacerraf et al., 1960; Dixon et al., 1961; Germuth et al., 1967; Princus et al., 1968). Moreover, the presence of antigen, probably in the form of antigen-antibody complexes, has been shown by immunofluorescence and electron microscopy in the glomeruli of animals (Dixon et al., 1961). Since the immune complexes deposited in the tissues were assumed to be in equilibrium with the soluble complexes in blood, immunologists have shown great interest in the detection and quantitative analysis of these soluble complexes in numerous diseases. This has been possible because of the development of various methods allowing the detection of complexes in a great variety of diseases ranging from the common cold to cancer and the so-called autoimmune diseases. Nevertheless, these investigations were unable

to define whether the complexes detected play a pathogenic role or are only the consequence of the disease.

Immune complexes tend to accumulate in the basal membranes (Cochrane, 1969; Gelfand et al., 1976) and, consequently, to cause various forms of vasculitis with, according to their location, a clinical presentation of glomerulonephritis (Cruchaud et al., 1975; Morris et al., 1975; Perrin et al., 1975), periarteritis (Gocke et al., 1970; Trepo and Thivolet, 1970), erythema nodosa (Hedors and Norberg, 1974; Sergent et al., 1976; Verrier-Jones et al., 1976; Zoller et al., 1978). They may also be deposited in the dermis where they can provoke urticaria (Marder et al., 1976; Lurhuma et al., 1976; Vinceneux, 1977), and in the articular cartilages with consequente arthritis (Winchester et al., 1970; Hurd et al., 1971; Natvig et al., 1974; Norberg et al., 1974; Russell et al., 1974; Rosenthal et al., 1976). By binding to the Fc receptors of the blood platelets, they may provoke thrombopenia (Miescher and Cooper, 1960; Marney and Des Prez, 1971; Penttinen et al., 1971; Osler and Siraganian, 1972; Israels et al., 1973). Finally, the binding of complexes to the membrane of some cells may result in the release of active substances. For example, neutrophile leucocytes will undergo degranulation with, as a consequence, the release of lysosomial hydrolases (Henson, 1971).

Inflammatory action and the elimination of complexes by macrophages are principally a function of the complement system (Müller-Eberhard, 1975), consisting of about a dozen serum proteins. Because of its central role and its high serum concentration, C3 factor is particularly important. It can be activated by the classical pathway, involving C1, C4 and C2, or by the alternative properdin pathway (Fig. 18). Immune complexes containing IgG and IgM activate complement via C1, whereas those with IgA act via properdin. The latter system can also be triggered directly by endotoxins and various polysaccharides in the absence of antibodies. Activation of C3 involves splitting the molecule into two fragments, a smaller one, called C3a, and a larger, C3b. Initiation of the activation of C5, C6 and C7 by factor C3b will lead to the lysis of the cellular antigen by C8 and C9. C3b can also be adsorbed onto the macrophage membrane and favours in this way the endocytosis of immune complexes. Fragment C3a is characterized by its ability to cause the degranulation of basophile leucocytes. Quantitative analysis of C3, showing a decrease of its serum level, can disclose a complement consumption, which is generally associated with deposits of immune complexes.

It must be noted that the occurrence of circulating immune complexes is often associated with autoimmune reactions and immunodeficiencies, such as those of complement factors, immunoglobulins and T-lymphocytes. Indeed, immune complexes could block immune response, for example in some cancers (Baldwin et al., 1972; Jose and Seshadri, 1974; Kilburn et al., 1976), and perhaps trigger autoimmune reactions, such as the production of rheumatoid factor (Williams and Kunkel, 1963). Moreover, autoantibodies can, as in case of lupus erythematosus, cause the occurrence of immune

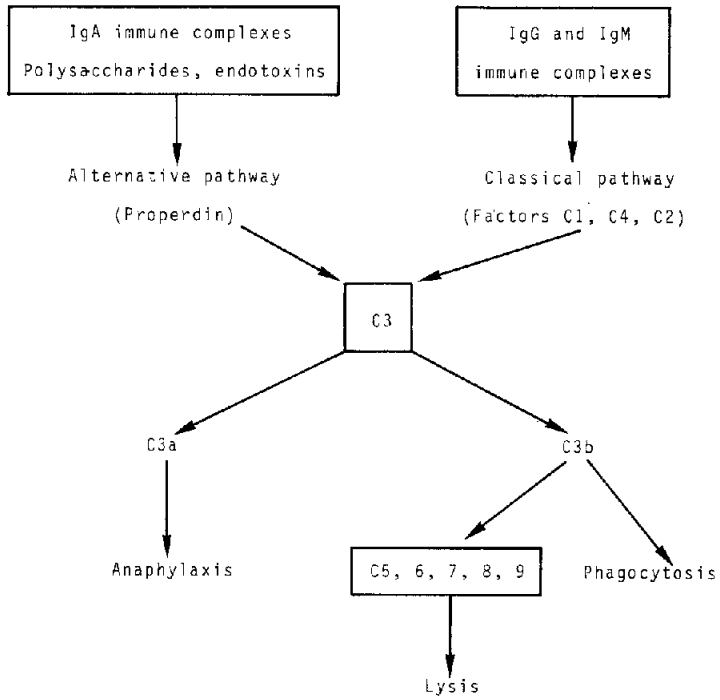

Figure 18. Complement fixation and its biological consequences.

complexes, for example DNA-anti-DNA complexes (Winfield et al., 1975) or inhibit the immune response when they are directed against T-lymphocytes (Nies et al., 1974).

Recent work (Howes and McKay, 1975) seems to indicate that immune complexes could play a role in the pathogenicity of uveitis. After intravenous injection of soluble immune complexes into the rabbit, the vascular permeability was found to be increased at the level of the ciliary processes and iris. Then, it may be asked whether the uveitis which occurs in patients with diseases associated with immune complexes in serum, is not due to the intraocular deposition of these complexes. These diseases are, for example, ankylosing spondylitis (Gabay et al., 1977), sarcoidosis (Hedfors and Norberg, 1974), Behçet's syndrome (Williams and Lehner, 1977; Gupta et al., 1978; Gamble et al., 1979) and Crohn's disease (Doe et al., 1973; Jewell and MacLennan, 1973).

Diseases of the uvea

To understand the pathogenic mechanisms of various diseases of the uvea and to find new diagnostic tests, we have determined the concentrations of immunoglobulins and C3 factor, and looked for the presence of immune

complexes in the AH and serum of various patients. Furthermore, we have studied the HLA antigens in 15 patients suffering from Fuchs' heterochromic cyclitis or Georgiades' syndrome, i.e. cataract-heterochromia of the iris.

The relative excess of IgG, IgA and IgM in the AH was defined by the relative concentration ratio (RCR) AH/S. The RCR for a given protein was obtained by dividing its AH/S concentration ratio by the same ratio for albumin. Thus, it can be determined for an individual patient, contrary to the relative clearance value, which must be calculated from data obtained from several patients. The RCR and relative clearance values will be essentially similar for the proteins whose transfer through the BAB is proportional to that of albumin. The RCR value for a protein will be higher than its relative clearance value if a certain amount of the protein concerned is, for example, synthesized in the eye. In practice, an immunoglobulin is in relative excess in the AH when its RCR value is equal to or higher than 0.65 for IgG; 0.60 for IgA and 0.40 for IgM. These values are the mean plus two standard deviations of the corresponding relative clearances (values b of Fig. 4). These criteria are applicable only for AH samples with a total protein level lower than 1 g/100 ml. Indeed, above this concentration, the proteins in the AH are in the same proportions as in serum (cfr. Chapter 1).

To detect immune complexes in the AH and serum, we used the agglutination-inhibition test. This test is based on the ability of the immune complexes to inhibit the agglutinating activity of rheumatoid factor (RF) or of subunit Q of the first component of complement (C1q) towards IgG-coated polystyrene particles (latex). The agglutination-inhibition tests were always performed with both agglutinating agents, because immune complexes, depending on their size and the class or subclass of immunoglobulins involved, do not necessarily react with RF and C1q in the same manner (Lurhuma et al., 1976). The RF reagent was the serum of a patient with rheumatoid arthritis having a latex agglutination titer of 1/320. C1q solution was given by the laboratory of Experimental Medicine. In practice, a volume of 25 μg of the sample was mixed on a dark plate with an equal volume of the RF or C1q solution, and then with 25 μl of the latex suspension. After 3 minutes, agglutination was scored positive or negative. For optimal sensitivity, RF and C1q solutions were used at their lowest concentration still causing distinct agglutination. Before testing the inhibiting properties, each sample was always checked for the possible presence of spontaneous agglutinating activity due to endogenous RF. The inhibiting or agglutinating activity of the samples was titrated by twofold serial dilutions.

Fuchs' heterochromic cyclitis. The RCR values for immunoglobulins were increased in a very variable manner in cases of various ocular diseases (Fig. 19). This was more often observed with IgG than with IgA or IgM. Among the different diseases, Fuchs' heterochromic cyclitis was most frequently

associated with a relative excess of IgG, in 28 out of 35 cases. Fuchs' cyclitis is an ocular disease which is generally unilateral, and is characterized by three principal signs: hypochromia of the iris, keratic precipitates, and cataract. Hypochromia is secondary to stromal atrophy, which begins in the pupillary area of the iris and is associated with hyalinosis of the vessel walls. Keratic precipitates are scattered on the whole posterior side of the cornea; they are small, star-shaped and translucent. They appear not to be lens proteins

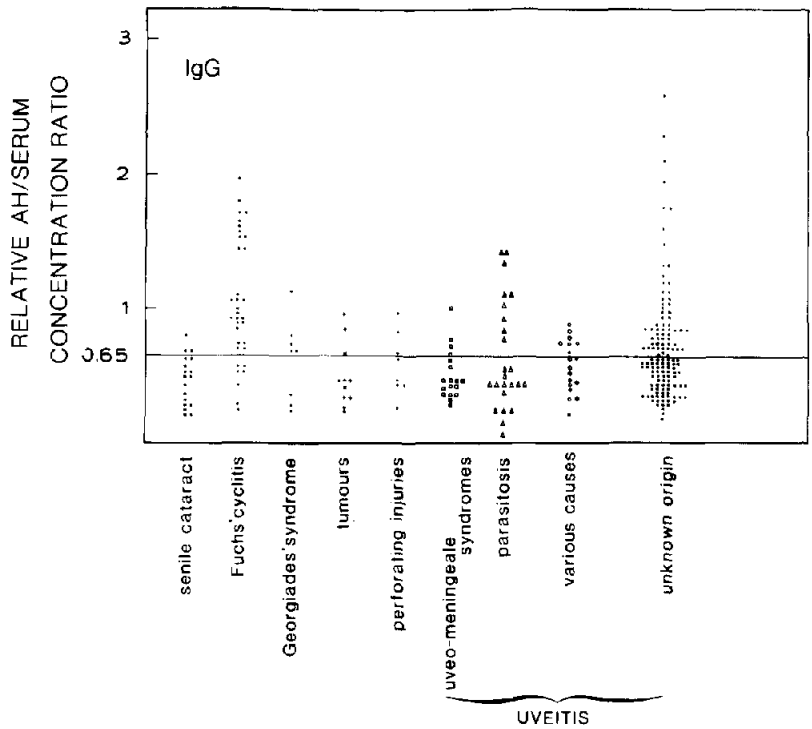

Figure 19. Relative AH/serum concentration ratios (RCR) for IgG, IgA and IgM in different ocular disorders. The RCR value for a given protein was obtained by dividing its AH/serum concentration ratio by that for albumin. Immunoglobulins are in relative excess in the AH when their RCR value is equal to or higher than 0.65 for IgG; 0.60 for IgA and 0.40 for IgM. These values are those of the corresponding relative clearances (values b of Fig. 4) plus two standard deviations.

+ = choroidal melanoma
× = retinoblastoma
□ = Behçet's syndrome
■ = Vogt-Koyanagi-Harada's syndrome
▲ = toxoplasmosis
○ = sarcoidosis
● = Crohn's disease (cf. various causes)
◇ = ankylosing spondylitis
◆ = scleroderma
△ = onchocerciasis

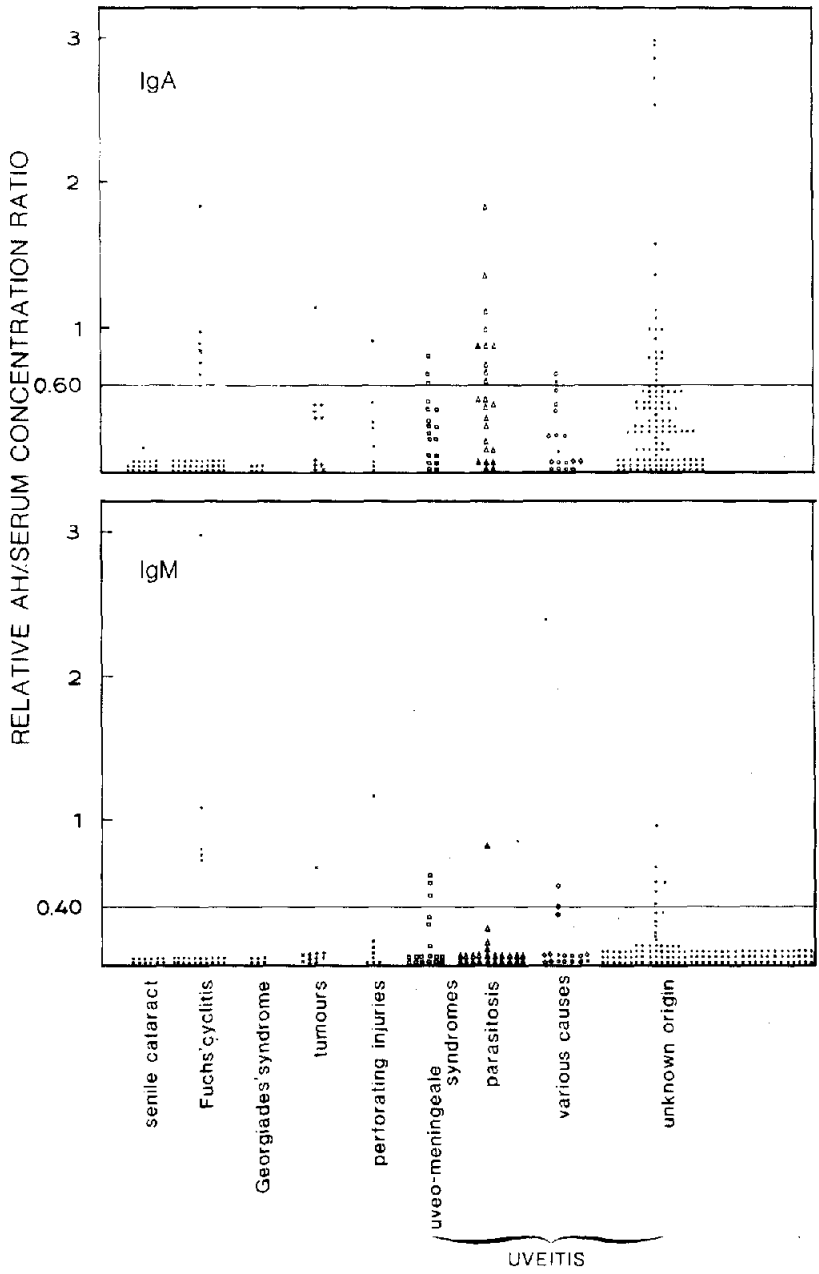

Figure 19. (Continued)

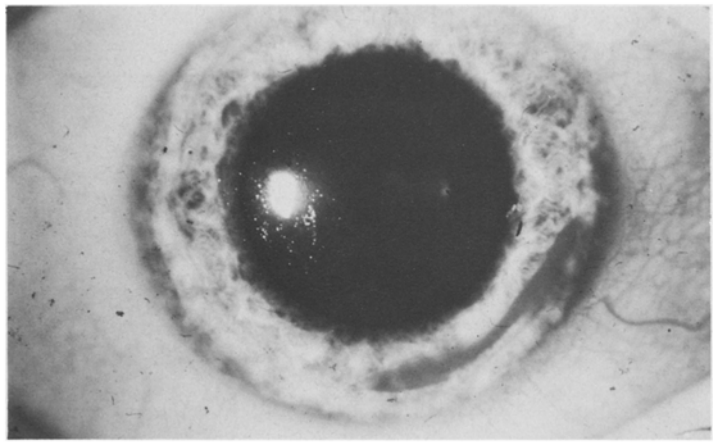

Figure 20. Filiform hemorrhage following puncture of the anterior chamber in a case of Fuchs' heterochromic cyclitis.

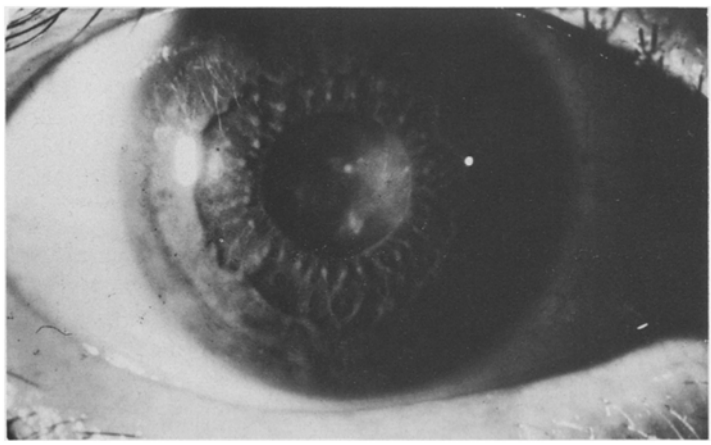

Figure 21. Nodules on the crests of the pupillary area of the iris in a case of Fuchs' heterochromic cyclitis.

because we have seen then reappearing after intracapsular extraction of the lens. They appear to be composed of lymphocytes and polymorphonuclear leucocytes (Verrey, 1974). Cataract appears later. It starts beneath the posterior capsule of the lens and is usually what causes the patient to seek medical advice. To this triad, one may add the filiform hemorrhage that follows puncture of the anterior chamber (Amsler and Verrey, 1946) (Fig. 20). This hemorrhage originates from the angle of the anterior chamber or from the root of the iris; it is discrete and present in nearly all our cases. It is not pathognomonic because it was observed in other ocular diseases such as, for example, sarcoidosic iridocyclitis. In some cases, we observed on the crests of the pupillary area of the iris little greyish nodules (Fig. 21) (Michiels and Dernouchamps, 1971) whose existence was previously reported, to our knowledge, only by Vogt (1952). The signification of these nodules remains unknown.

The relative excess of immunoglobulins in the AH could be the expression of immune reactions directed against a viral or bacterial agent or against the ocular tissues themselves. The infectious origin was suggested by Fuchs (1906), in connection with tuberculosis. Bacteria have been detected in the AH from some patients (Verrey, 1954; Offret et al., 1955), but it would be necessary to search for the presence of virus by means of more sensitive immunochemical methods and electron microscopy, as suggested by Witmer (1976) for other forms of uveitis. In other respects, antiuvea autoantibodies have been detected in the AH at particularly high titers, i.e. 70 times as much as in the serum (Remky, 1965b). Degradation of the uveal tissue following an infection or neurotrophic problems could be the cause of immune reactions against this altered tissue. A disturbance of the cervical sympathetic nervous system, suspected by Bistis (1912), has been found in 50% of the cases of Fuchs' disease (François, 1949; Michiels, 1968) by means of the benzedrine eye drops test, originally devised by Appelmans and Forez (1949). This could explain the unilaterality of the disease. However, this unilateral character of the Fuchs' disease seems inconsistent with the existence of autoimmune reactions. It could be assumed that autoantigens are accessible to antibodies and lymphocytes in one eye only, because of, for example, local infection. In other respects, the possible hereditary origin of a disease of the cervical sympathetic nerve could explain the development of Fuchs' disease in several members of one and the same family (Bistis, 1912; Makley, 1956; François, 1961).

Concerning the study of immune complexes, inhibitory or agglutinating factors were detected in 22 out of 28 AH samples examined (Table 2). On the other hand, our results were negative in 20 AH samples taken from patients with senile, immature cataract. Since this disease does not seem to involve immune reactions, these 20 samples may be considered as controls for our study.

Table 2. Titration of agglutinating or inhibiting factors in AH and serum from patients with Fuchs' heterochromic cyclitis

Patient No.	Date of sample collection	Agglutinating factors		Inhibiting factors vs. RF		Inhibiting factors vs. C1q	
		AH	Serum	AH	Serum	AH	Serum
1		0	0	1/8	1/2	1/2	0
2		0	0	0	0	0	0
3		0	0	0	0	0	0
4		1/1600	1/160	–	–	–	–
5	Dec. 1973	1/16	1/320	–	–	–	–
	Aug. 1974	0	0	1/1	1/1	1/1	1/4
	Jul. 1975	0	0	1/1	1/1	0	0
6	Nov. 1974	1/64	0	–	0	–	–
	Jul. 1975	0	1/32	1/2	–	1/2	–
	Jun. 1976	0	0	1/2	0	1/1	0
7	Jul. 1975	0	1/80	1/1	–	–	–
	Apr. 1976	0	0	1/2	0	1/2	0
8	Jul. 1974	1/1	1/640	–	–	–	–
	Aug. 1975	0	0	0	0	1/2	0
9		1/8	1/40	–	–	–	–
10		1/1	1/20	–	–	–	–
11		0	0	1/4	0	1/2	0
12		1/2	1/16	–	–	–	–
13		0	0	0	1/1	1/4	0
14		0	0	1/1	0	–	0
15		0	0	0	0	0	0
16		0	0	0	0	1/1	0
17		0	0	0	0	0	0
18		0	1/4	0	–	1/1	–
19		0	0	0	0	0	0
20	Oct. 1976	1/1	1/8	–	–	–	–
	Nov. 1977	0	1/8	0	–	0	–
21		0	0	0	0	1/2	0

Could the inhibitory activity of the AH samples correspond to other factors than immune complexes or aggregated IgG? Among the possible interferring factors, single stranded DNA might inhibit C1q (Agnello et al., 1970), but the fact that of our 14 AH samples displaying an inhibitory activity, 6 were found to inhibit both C1q and exogenous RF, indicates that the inhibitory factors do correspond, in these cases, to immune complexes or aggregated immunoglobulins.

We have previously demonstrated that the agglutinating factor of AH and serum samples is probably IgM (Dernouchamps et al., 1977) and, thus, RF. In the AH samples taken at different intervals during the evolution of Fuchs' disease, inhibitory activity was found to alternate with spontaneous

agglutinating activity. Is this agglutinating activity secondary to the presence of immune complexes? This has been suggested by Williams and Kunkel (1963) following experiments where the production of RF was induced in rabbits by injecting immune complexes or aggregated autologous IgG. On the other hand, it is well known that IgM-RF, which can be detected by the agglutination reaction, is generally associated with IgG-RF, devoid of agglutinating activity. This IgG-RF has a great tendency to combine with other IgG molecules, themselves displaying RF-activity. The result is the formation of IgG-IgG complexes (Fig. 22), which are in fact immune complexes (Pope et al., 1974). Thus, it is not surprising that, after elimination of agglutinating RF, one may detect inhibitory factors corresponding probably to this kind of IgG-RF immune complexes (Lurhuma et al., 1977).

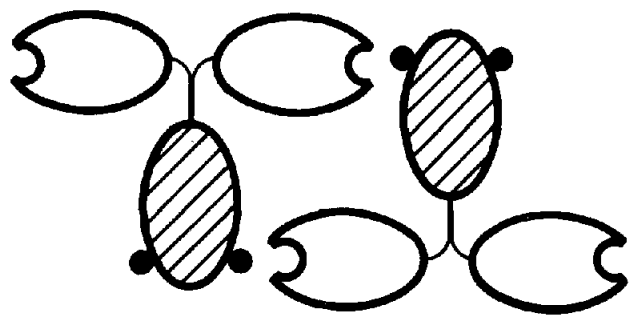

Figure 22. IgG-IgG immune complexes, where IgG with a rheumatoid factor activity acts both as antigen and antibody. The hatched area represents the Fc fragment of an IgG molecule, while the non-hatched areas represent the Fab fragments. The Fc fragment is responsible for various biological properties of IgG such as, for examples, complement fixation; the Fab fragments are responsible for the antibody activity of IgG. (Drawing reproduced from Pope et al., Proc. Nat. Acad. Sci. USA, 71: 517–521, 1974 with kind permission of Dr. Pope).

It may be asked whether the inhibitory factors in the AH do not correspond to IgG aggregates which were formed during storage of the samples. This phenomenon is known to occur in biological fluids which are relatively rich in IgG and poor in albumin (Soltis et al., 1979). Against this hypothesis is the fact that no correlation exists between the RCR for IgG and the inhibitory activity.

A local origin for aqueous RF and immune complexes is likely since these factors were detected in the AH and not in the serum, or at the same titer in both fluids, although there were large differences in their protein content.

In addition to IgG which can play the role of both antigen and antibody, it is likely that other antigens of viral or bacterial origin can take part in the formation of immune complexes. Moreover, one must take into account the possibility of autoantigens such as uvea, lens or retina.

Immune reaction seemed to be less severe in the AH of patients with cataract-hypochromia without keratic precipitates, i.e. Georgidades' syndrome (1956). The RCR value for IgG was slightly increased in 5 of our 9 patients (Fig. 19), while RF was detected in samples of AH from 2 patients, at titers of 1/16 and 1/1. The mean age of our patients with Georgiades' syndrome was 59 years (SD: 13 years) and of those with Fuchs' cyclitis it was 31 years (SD: 13 years). Since histopathological lesions of the iris are similar in both diseases (Georgiades, 1956), it may be asked whether Georgiades' syndrome is not merely Fuchs' cyclitis where the immune reactions have been attenuated by age.

Although both syndromes seem to be associated with immune disorders, the study of HLA groups disclosed no significant deviation of the normal frequency distribution (De Bruyère, 1978). However, it must be noted that this study concerned only 15 patients, 11 with Fuchs' cyclitis and 4 with Georgiades' syndrome.

Endogenous uveitis. From the study of patients with endogenous uveitis, it appeared that the RCR values for immunoglobulins were less often increased than in cases of Fuchs' cyclitis (Fig. 19). But the frequency of finding elevated RCR values was approximately the same, whatever the origin of the endogenous uveitis. Thus, these results did not give information suitable for differential diagnosis. Consequently, the quantitative determination of immunoglobulins seems useful in clinical practice only when it is combined with that of specific antibodies in order to calculate a value for an antibody quotient.

Factors agglutinating latex or inhibiting C1q or RF have been detected in 43 AH samples out of the 104 examined (Table 3). In general (38/43), these factors were restricted to the AH or reached about the same titer in the AH and in the serum. That suggests an intraocular formation of these factors. In addition, our tests were positive in the serum of 52 patients. In 26 of these patients, AH was free of immune complexes or RF. Detection of immune complexes or RF was especially common in the serum of patients suffering from Behçet's disease (11 out of 14) and in the AH of patients with an ocular onchocerciasis (9 out of 13). In the latter disease, no correlation was observed between the presence of these factors in the AH and the various clinical aspects: punctate keratitis, iridocyclitis, Ridley's chorioretinitis or the presence of microfilariae in the anterior chamber.

In clinical practice, the presence of immune complexes or RF in the AH has not been shown to be specific for a given form of endogenous uveitis. However, it raises the question of the possible role of immune complexes in the intraocular inflammation. As a result of their deposition in the different ocular tissues, immune complexes can induce various inflammatory reactions, e.g. by complement fixation. Since complement fixation can be manifested by a decreased concentration of C3 factor, the concentration of C3 was

Table 3. Occurrence of agglutinating or inhibiting factors in AH and serum from patients with endogenous uveitis

		Number of cases	Agglutinating factors		Inhibiting factors	
			AH	Serum	AH	Serum
Uveo-meningeal syndromes	Vogt-Koyanagi-Harada	3	2	2	0	0
	Behçet	14	1 + 1*	9	1*	2
Parasitosis	Onchocerciasis	13	2	7	3 + 4***	0
	Toxoplasmosis	3	0	1	0	1
Various causes	Sarcoidosis	1	0	1	0	0
	Crohn	2	0	1	0	0
	Ankylosing spondylitis	4	1 + 1**	1	1***	1
	Scleroderma	1	1*	1	0	0
Unknown origin		63	12 + 2*	22	8 + 3***	3
Total		104	23	45	20	7

*titer lower than in serum
**associated with the presence of inhibiting factors in serum
***associated with the presence of agglutinating factors in serum

determined in the AH and serum of 47 patients suffering from uveitis. The RCR values for C3 were found to be very scattered (Fig. 23) in the patients with agglutinating or inhibiting factors in the AH as well as in the patients without such factors. Moreover, it must be noted that the mean value for RCR was the same ($\bar{X} = 0.25$) for both groups of patients, whereas one would expect C3 to be consumed by complement fixation in patients with immune complexes. From this apparent lack of complement fixation, it may be asked whether complement can be activated in the AH. Indeed, the various factors of complement are probably not in the same proportion in the AH as in the serum, because of the differences between their molecular weights and, consequently, their ability to cross the BAB.

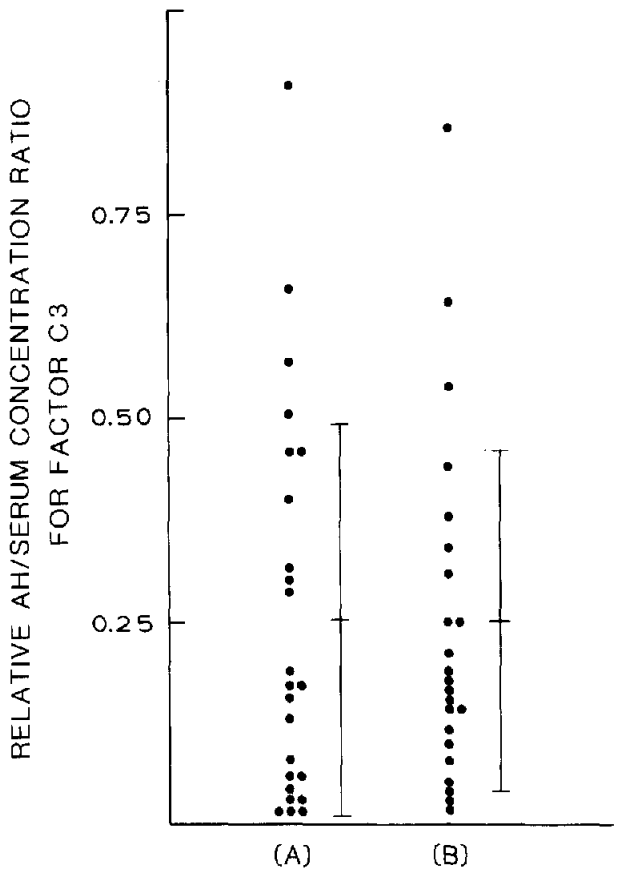

Figure 23. Relative AH/serum concentration ratio (RCR) for the C3 factor of complement in 47 patients with uveitis. Immune complexes or rheumatoid factor were detected in the AH samples from 24 of these patients (group A) but not in those from the other 23 (group B). The RCR value for a protein was obtained by dividing its AH/serum concentration ratio by that for albumin. For each group, the bar correponds to the mean of the RCR values ± 1 standard deviation.

It is well known that posterior subcapsular cataract generally develops in case of chronic uveitis. Therefore, we searched for a possible correlation between this form of cataract and the presence of agglutinating or inhibiting factors in the AH of 54 patients suffering from uveitis, generally of unknown origin. This correlation was found to be at the limit of significance ($0.05 > P > 0.02$) (Table 4). However, Fuchs' cyclitis, which is associated in 80% of

Table 4. Correlaton between the presence in AH of inhibiting or agglutinating factors and the development of secondary cataract in 54 patients suffering from uveitis, usually of unknown origin ($\chi^2 = 4.71$; $0.05 > P > 0.02$)

	Presence of inhibiting or agglutinating activity in AH		
	Yes	No	Total
Secondary cataract	17	9	26
No secondary cataract	9	19	28
Total	26	28	54

the cases with the presence of immune complexes or RF in the AH, is also characterized by the same posterior subcapsular cataract. This suggests that the development of cataract is due to deposition of immune complexes on the lens capsule. Recent work (Eeckhout et al., 1976) demonstrating a characteristic affinity of collagen for aggregated immunoglobulins could explain the preferential deposition of complexes on the capsule. In other respects, the presence of immune complexes could be due to the release of lens autoantigens. Finally, a third hypothesis could be the absence of a causal relationship between both phenomena, which could have a common origin such as, for example, a viral infection.

Intraocular tumours. It is generally accepted that patients with a cancer develop immune reactions against their own tumour. For example, skin tests, carried out with a soluble extract of malignant melanoma, were positive in 90 percent of cases of choroidal melanoma (Char, 1978). These reactions could explain why malignant melanoma can be apparently confined to the eye for many years before metastasing into the liver, kidneys and lungs. However, skin tests were also positive in 18.5 percent of the reference population. In case of ocular melanoma already diagnosed by ophthalmoscopy, ultrasonography and fluorescein angiography, these immune reactions could give information about the prognosis, because they have been found to be reduced in case of extraocular metastasis (Char, 1978; Priluck et al., 1979). This depression could be due to the action of the so-called blocking factors (Hellström et al., 1969–1971; Diehl et al., 1971; Bansal et al., 1973), corresponding perhaps to circulating immune complexes (Baldwin et al., 1972; Jose

and Seshadri, 1974; Kilburn et al., 1976) which are frequently detected in the serum of patients with cancer.

Analysis of the AH from 8 patients with choroidal melanoma disclosed a relative excess of IgG in 2 cases and agglutinating or inhibiting factors in 3 other cases (Table 5). No correlation was found between these immune reactions and the histological type of the tumour. However, it must be noted that the 3 patients with agglutinating or inhibiting factors in their AH had a retinal detachment around the tumour. Moreover, in 2 of these 3 patients, the presence of tumour cells along intrascleral vessels suggested metastases. However, the number of patients was too small to allow statistically significant conclusions. The same is true of those patients with retinoblastoma having immune complexes in their serum (Char et al., 1978) and AH (Table 5).

Ocular perforating injuries. Sympathetic ophthalmia is a severe uveitis following ocular perforating injury with transmission to the heterolateral eye. It is occasionally associated with neuro-encephalitic signs, such as a discrete meningeal syndrome, hypoacousia or encephalitis, and with cutaneous manifestations, such as alopecia and vitiligo. Bilateralization of the uveitis seems to be due to immune reactions directed against an infectious agent or against the ocular tissues themselves, such as uvea (Woods, 1921, McPherson and Woods, 1948; Aronson et al., 1966) or retina (Marak, 1973).

After ocular perforating injury, the RCR values for the immunoglobulins were found to be normal or slightly increased (Fig. 19). No difference was observed in the cases developing sympathetic ophthalmia. Moreover, RF or immune complexes were found in the AH samples from 3 patients out of 6 examined. Details of the case histories of these 3 patients are interesting.

Case 1: S . . . Raoul was treated for a perforating injury of the right eye by an iron foreign body. This was extracted within 24 hours. As there was still irritation of the wounded eye one month later, an aqueous puncture was carried out. The AH sample obtained was clear, but contained RF at a titer of 1/5120. A serum sample, taken at the same time, contained neither RF nor immune complexes. As the perforated eye was blind, enucleation was recommended to the patient, who refused it. Sympathetic ophthalmia developed 15 days after the examination.

Case 2: the right eye of B . . . Alain appeared irritated, 2 years after a perforating injury. An aqueous puncture was carried out. The AH sample contained RF at a titer of 1/8, while his serum was negative. The perforated eye, which was sightless, was enucleated. Histopathological analysis showed an infiltration by lymphocytes and plasmocytes suggesting a sympathetic opthalmia.

Case 3: the left eye of G . . . Raymond, which remained irritated for 10 years after a perforating injury was removed. The diagnosis of sympathetic

Table 5. Titration of agglutinating or inhibiting factors in AH and serum in cases of choroidal melanoma (patients 1 to 8) and retinoblastoma (patients 9 to 11)

Patient No	Histological type	Inflammatory retinal detachment	Risk of metastasis*	Agglutinating factors		Inhibiting factors		Relative excess of Ig in AH
				AH	Serum	AH	Serum	
1	Spindle cell A	0	0	0	0	0	0	0
2	Spindle cell B	+	0	1/4	0	–	0	0
3	Spindle cell A	0	0	0	0	0	1/4	IgG
4	Spindle cell A	0	0	0	0	0	0	0
5	Epithelioid	+	+	0	1/8	1/4	–	0
6	Spindle cell B	+	+	0	0	1/1	1/2	0
7	Spindle cell A	+	+	0	0	0	0	0
8	?	0	0	0	1/2	0	–	IgG
9	Differentiated	?	0	0	0	0	0	0
10	?	?	0	0	0	0	0	0
11	Undifferentiated (necrotic)	?	+	1/16	0	–	0	IgG-IgA-IgM

*The risk of metastasis can be defined histopathologically by the presence of tumour cells along intrascleral vessels in cases of choroidal melanoma and in the optic nerve in cases of retinoblastoma

ophthalmia was then established histopathologically. Two months later, a total uveitis developed in the other eye, with immune complexes at a titer of 1/1 in the AH, and RF at a titer of 1/16 in the serum.

Thus it must be noticed that the three patients with RF or immune complexes in their AH developed a sympathetic ophthalmia. On the other hand, the tests were negative in the AH of the 3 other patients who developed respectively an ocular siderosis, an atrophy of the eye and an ossification of the choroid, but without inflammatory reaction threatening the other eye. The probability that the association between sympathetic ophthalmia and the presence, in the AH, of RF or immune complexes could have arisen by chance was 0.05 (Exact probability test of Fisher).

General conclusions

1. The determination of the total protein content of the aqueous humour (AH) provided information about the intensity of the uveal inflammation which was more accurate than that obtained from measurements of the Tyndall effect with a slit-lamp. Nevertheless, an ocular puncture would not be justifiable, if the analysis of the aqueous humour were to be limited to measurement of the total protein content.

2. The concentration ratios AH/serum for various proteins were inversely proportional to the molecular weight of the proteins studied, except in the group of patients with a total AH protein level higher than 1 g/100 ml, where no size restriction was observed. Using these data, a mean value has been calculated for the size of the hypothetical pores allowing serum proteins to pass across the blood-aqueous barrier. Our data proved to be compatible with the assumption that the blood-aqueous barrier behaves as an isoporous membrane with a pore radius of about 104 Å for the groups of patients with an AH protein level lower than 125 mg/100 ml, and about 113 Å for the patients having an AH protein content between 125 and 1000 mg/100 ml.

3. Transferrin was found to be in small excess, relative to albumin, in the aqueous humour of humans, rabbits and guinea pigs. A great abundance of this glycoprotein was noted in the vitreous humour of rabbits. Electrophoretic analyses indicated that, in rabbits, the transferrin of the aqueous humour migrated in exactly the same manner as that of the serum. On the other hand, vitreous humour transferrin appeared to move in three bands, perhaps as a result of differences in sialic acid content. Study of the specific activity of labelled transferrin after intravenous injection into rabbits suggests that the relative abundance of transferrin in the intraocular fluids may result from a local biosynthesis in the posterior segment of the eye. The bacteriostatic properties of transferrin could help maintain sterility in the intraocular media.

4. A relative excess of IgG was found in the aqueous humour of patients suffering from Fuchs' heterochromic cyclitis (in 28 cases out of 35 examined).

In cases of other ocular disorders, the relative concentration ratio (RCR) for immunoglobulins was less frequently increased. Thus, the determination of the concentration of immunoglobulins in the aqueous humour seems useful in clinical practice to confirm the diagnosis of Fuchs' cyclitis. In cases of other uveal diseases, these quantitative determinations seem useful only when combined with those of specific antibodies in order to calculate a value for an antibody quotient.

5. The presence of immune complexes or RF in the AH has not been shown to be specific for a given ocular disease. However, it could be interesting for the prognosis. Indeed, the presence of immune complexes or RF in the AH could occur before development of secondary cataract in cases of uveitis, sympathetic ophthalmia after performating ocular injury and, perhaps, metastasis in cases of intraocular tumour.

Acknowledgements

The present work was performed at the University Eye Clinic (Head: Prof. J. Michiels) of the Université Catholique de Louvain and at the Unit of Experimental Medicine (Head: Prof. P.L. Masson) of the Université Catholique de Louvain and the International Institute of Cellular and Molecular Pathology.

I am indebted to Professors J. Michiels and P.L. Masson for helpful discussions.

I thank Mrs. Tsai-De Kempeneer for illustration of this monograph, and Dr. A Leek for kindly correcting the manuscript typed by Mrs. Joris-Dessy.

Résumé

Pour le dosage des protéines totales de l'humeur aqueuse (HA), nous avons adopté la méthode de Meulemans. Celle-ci est basée sur une précipitation à l'acide trichloracétique avec lecture turbidimétrique. Nos résultats, chez des patients avec cataracte sénile, immature, sont semblables à ceux obtenus par le micro-Kjeldahl, méthode de référence, qui nécessite un temps d'exécution fort long. Les taux de protéines totales dans l'HA augmentent de manière fort variable dans les différentes affections oculaires, sauf en cas de cyclite hétérochromique de Fuchs où aucune valeur supérieure à 80 mg/ml n'est observée. Les données obtenues par ces dosages sont plus précises que celles fournies par la mesure de l'effect Tyndall au moyen de la lampe à fente. Toutefois, une ponction de chambre antérieure ne paraît pas justifiée si l'analyse de l'HA se limite au dosage des protéines totales.

Pour les dosages spécifiques de protéines, nous avons choisi l'immunodiffusion radiaire. Celle-ci nous a permis de doser 7 protéines dans l'HA et le sérum de 109 patients. Les résultats des dosages ont été répartis en quatre groupes selon le taux des protéines totales de l'HA. Si l'on ne considère que

les protéines, l'HA normale peut être assimilée à un ultrafiltrat du sérum. Aussi, exprimons-nous les résultats sous la forme d'un rapport des concentrations dans l'humeur aqueuse et dans le sérum (HA/S). Ceci neutralise les variations du taux des protéines dans le sérum. Comme on pouvait s'y attendre, les rapports HA/sérum pour chacune des protéines augmentent avec le taux des protéines totales dans l'HA. Confirmant la notion de filtration, les mêmes rapports varient en fonction inverse du poids moléculaire des protéines, sauf dans le groupe de patients avec un taux de protéines totales supérieur à 1 g pour 100 ml d'HA, où aucune restriction au passage n'est observée. Une exception retient l'attention: le rapport HA/sérum pour la transferrine est plus élevé que ne le fait prévoir le poids moléculaire de cette protéine.

En utilisant les informations fournies par les dosages spécifiques de diverses protéines dans l'HA et le sérum, une valeur moyenne a été calculée pour la taille des pores hypothétiques permettant le passage des protéines à travers la barrière hémato-camérulaire. Pour effectuer ces calculs, il fallait tenir compte des variations individuelles des rapports HA/sérum et de l'abondance relative de transferrine dans l'HA. C'est la raison pour laquelle, dans chaque groupe de patients, nous avons considéré le rapport HA/sérum pour chaque protéine en fonction du rapport correspondant pour l'albumine, protéine de référence. Ces corrélations sont apparues de type linéaire. Dans les équations de la régression ($Y = Yo + bX$), les termes constants (Yo) n'étaient généralement pas significatifs ($P > 0{,}05$), sauf pour la transferrine ($P < 0{,}0001$) dans les deux groupes de patients avec un taux de protéines totales dans l'HA inférieur à 125 mg/100 ml, et pour l'IgG ($P = 0{,}0001$) dans le groupe de patients avec un taux de protéines dans l'HA situé entre 50 et 125 mg/100 ml. L'existence d'une valeur significative de Yo signifie qu'une certaine quantité de la protéine concernée accède à l'HA indépendamment du passage de l'albumine à travers la barrière hémato-camérulaire. Les coefficients de régression (b) pour les différentes protéines étaient inversement proportionnels au poids moléculaire des protéines en question. Ils peuvent être assimilés aux valeurs de clearance relative pour les différentes protéines.

En utilisant les équations de Pappenheimer, on a trouvé une relation théorique entre le clearance relative de diverses protéines et leur rayon de diffusion moléculaire. Au total, nos données se sont révélées compatibles avec l'hypothèse que la barrière hémato-camérulaire se comporte comme une membrane isopore avec un rayon de pore d'environ 104 Å pour les groupes de patients avec un taux de protéines totales dans l'HA inférieur à 125 mg/100 ml et d'environ 113 Å pour les patients ayant un taux de protéines situé entre 125 et 1000 mg pour 100 ml d'HA.

Comme il était impossible, chez l'homme, d'obtenir un grand nombre d'échantillons d'HA 'normale' et d'effectuer certaines expériences, nous avons étudié la transferrine chez l'animal. Des dosages dans les liquides

endoculaires et dans le sérum ont révélé un léger excès de transferrine, relativement à l'albumine, dans l'HA du lapin (n = 39) et due cobaye (n = 14), et une grande abondance de cette glycoprotéine dans le vitré du lapin (n = 18).

L'étude de la transferrine, chez le lapin, a porté d'abord sur certaines de ses propriétés dans des pools concentrés d'humeur aqueuse et de vitré. Les résultats montrent que la transferrine, dans ces deux liquides endoculaires, possèdent tous les déterminants antigéniques, présents dans la transferrine du sérum. En outre, des études électrophorétiques combinées à des analyses enzymatiques semblent indiquer que, chez le lapin, la transferrine du sérum et celle de l'HA, ainsi qu'une grande partie de la transferrine du vitré contiennent deux résidus d'acide sialique par molécule. Quant au reste de la transferrine du vitré, il serait dépourvu d'un ou des deux résidus d'acide sialique normalement présents dans cette glycoprotéine. Par ailleurs, nous avons tenté d'expliquer l'accumulation endoculaire de transferrine. Celle-ci pourrait résulter d'un transport actif à travers la barrière hémato-camérulaire, d'une concentration sélective suite à une absence d'élimination, ou encore d'une synthèse locale. C'est pourquoi, nous avons déterminé, dans les différents compartiments de l'oeil, la présence de transferrine hétérologue et l'activité spécifique de transferrine homologue, marquée à l'^{125}I, après injection de ces deux protéines dans la veine marginale de l'oreille du lapin. Ces études suggèrent la possibilité d'une synthèse locale de transferrine au niveau du pôle postérieur de l'oeil.

En pratique clinique, les dosages de protéines dans l'HA ne paraissent utiles que lorsqu'ils concernent les immunoglobulines. Dans cette perspective, la présente étude a été limitée au dosage spécifique d'immunoglobulines et à la recherche de complexes immuns dans l'HA et le sérum de patients atteints de diverses affections de l'uvée.

Pour rechercher un excès relatif d'IgG, IgA et IgM dans l'HA, les rapports de concentration relatifs (RCR) HA/sérum pour ces immunoglobulines ont été comparés à leur clearance relative correspondante. Le RCR pour une protéine donnée s'obtient en divisant son rapport de concentration HA/sérum par celui de l'albumine. Il peut donc être déterminé chez un seul malade, contrairement à la clearance relative qui ne peut être calculée qu'à partir des données provenant de plusieurs patients. La valeur de RCR pour une protéine sera supérieure à celle de sa clearance relative, lorsqu'une certaine quantité de la protéine concernée est, par exemple, synthétisée dans l'oeil. En pratique, une immunoglobuline est en excés relatif dans l'HA lorsque son RCR est égal ou supérieur à 0,65 pour l'IgG; 0,60 pour l'IgA et 0,40 pour l'IgM. Ces critères ne sont toutefois valables que pour les HA avec moins de 1 g de protéines totales pour 100 ml, car, au-delà de cette concentration, les protéines dans l'HA sont dans la même proportion que dans le sérum.

Pour détecter les complexes immuns, nous avons utilisé une méthode basée sur la propriété des complexes d'inhiber l'activité agglutinante du facteur rhumatoïde (FR) ou de la sous-unité C1q du premier facteur du

complément vis-à-vis de particules couvertes d'IgG humaines (latex). Les tests d'inhibition d'agglutination ont été effectués avec le FR et avec le C1q, parce que ces deux agents agglutinants n'ont pas le même spectre d'activité en ce qui concerne la taille des complexes et la classe ou sous-classe d'immunoglobuline qui les compose. Les complexes immuns sont fréquemment associés à la présence de FR endogène. Aussi, avant la recherche des propriétés inhibantes, chaque échantillon a été testé en vue de déceler une activité agglutinante possible liée à la présence de FR endogène.

Parmi les 255 patients atteints de diverses affections oculaires (cyclite de Fuchs, uvéite endogène, tumeur intraoculaire, plaie perforante du globe), l'excès relatif d'immunoglobulines dans l'HA s'observe avec une fréquence équivalente, quelle que soit l'origine de l'affection, sauf en cas de cyclite de Fuchs où les valeurs de RCR pour l'IgG ont été trouvées augmentées dans 28 des 35 échantillons d'HA examinés. Ces résultats ne permettent donc pas de diagnostic différentiel, sauf peut-être en cas de cyclite de Fuchs. Aussi, les dosages d'immunoglobulines ne paraissent-ils intéressants en clinique que s'ils sont combinés à des dosages d'anticorps spécifiques permettant d'établir un quotient d'anticorps.

La présence de complexes immuns ou de FR dans l'HA n'a pas été trouvée spécifique d'une affection oculaire donnée. Elle pourrait toutefois présenter un intérêt pronostique. En effet, la présence de complexes immuns ou de FR dans l'HA semble précéder l'apparition de cataracte compliquée en cas d'uvéite, l'ophtalmie sympathique après perforation du globe oculaire et, peut-être, l'essaimage en cas de mélanome de la choroïde.

References

Aasa R, Malmstrom BG, Saltman P and Vänngärd T (1963) The specific binding of iron and copper to transferrin and conalbumin. Biochim Biophys Acta 75:203–222

Agnello V, Winchester RJ and Kunkel HG (1970) Precipitin reactions of the C1q component of complement with aggregated γ-globulins and immune complexes in gel diffusion. Immunology 19:909–919

Aisen P (1970) Physics and physiology of transferrin. Mt Sinaï J Med 37:213–222

Aisen P, Leibman A and Reich HA (1966) Studies on the binding of iron to transferrin and conalbumin. J Biol Chem 241:1666–1671

Alaerts L (1948a) L'humeur aqueuse, élément diagnostic. Acta Med Belg, Bruxelles

Alaerts L (1948b) Fluctuation du taux d'albumine dans l'humeur aqueuse. Bull Soc Fr Ophtalmol 81:349–355

Allansmith MR, Whitney CR, McClellan BR and Newman LP (1973) Immunoglobulins in the human eye – location, type and amount. Arch Ophthalmology 89:36–45

Allison AC (1977) Autoimmune diseases: concepts of pathogenesis and control. In: Talal (ed), Autoimmunity. New York, Academic Press p 91–139

Amsler M and Verrey F (1946) Hétérochromie de Fuchs et fragilité vasculaire. Ophthalmologica 111:177–181

Appelmans M and Forez J (1949) Effets pupillaires du sulfate de bënzédrine. Bull Soc Belg Ophtalmol 91:168–175

Appelmans M and Michiels J (1947) Technique expérimentale pour l'étude de la barriére hémato-camérulaire. Bull Soc Belg Ophtalmol 87:90–93

Aronson S, Schnellmann DC and Yamamoto EA (1966) Uveal auto-antibody in ocular diseases. J Am Med Assoc 196:225–228

Ashton N and Cunha-Vaz JG (1965) Effect of histamine on the permeability of the ocular vessels. Arch Ophthalmology 73:211–223

Baker E, Shaw DC and Morgan EH (1968) Isolation and characterization of rabbit serum and milk transferrins. Evidence for difference in sialic acid content only. Biochemistry 7:1371–1378

Balavoine C and Vuataz N (1949) Microdosage de l'azote protéinique dans l'humeur aqueuse. Ophthalmologica 118:356–368

Baldwin RW, Price MR and Robins RA (1972) Blocking of lymphocyte-mediated cytotoxicity for rat hepatoma by tumour-specific antigen-antibody complexes. Nature New Biol 238:185–186

Bansal SC, Hellström, KE, Hellström I and Sjögren HO (1973) Cell-mediated immunity and blocking serum activity to tolerated allografts in rat. J Exp Med 137:590–602

Benacerraf B, Potter JL, McClusky RT and Miller F (1960) The pathologic effects of intravenously administered soluble antigen-antibody complexes. J Exp Med 111: 195–200

Berkallof A, Bourguet J, Favard P and Lacroix JC (1977) Biologie et physiologie cellulaires. I. Membrane plasmique, etc. Paris, Hermann

Bistis J (1912) La paralysie du sympathique dans l'étiologie de l'hétérochromie. Arch Ophthal 32:578–583

Böke W (1965) Uveitis and allergy. Int Ophthalmol Clin 5:755–788

Bossak HN, Rosenberg AA and Harris A (1949) A quantitative turbidimetric method for the determination of spinal fluid protein. J Vener Dis Inform 30:100–103

Brambell FWR, Hemmings W, Oakley F and Porter R (1960) Relative transmission of fractions of papain hydrolized homologous γ-globulin from uterin cavity to foetal circulation in the rabbit. Proc Roy Soc Biol 151:478–482

Brewerton DA, Nichols A, Caffrey M, Walters D and James DCO (1973) Acute anterior uveitis and HLA-27. Lancet 2:994–996

Brown WR, Isobe K, Nakane PK and Pacini B (1977) Studies of translocation of immunoglobulins across intestinal epithelium. IV. Evidence for binding of IgA and IgM to secretory component in intestinal epithelium. Gastroenterology 73:1333–1339

Bruns RR and Palade GE (1968) Studies on blood capillaries. I. General organization of muscle capillaries. J Cell Biol 37:244–276

Cagianut B (1957) Elektrophorese des pathologischen Kammerwassers. Doc Opthalmol 11:134–137

Campinchi R, Denis-Lassale J, Virat J, Marsetio M and Offret G (1967) Apport de l'immunologie au diagnostic de l'herpès oculaire. Bull Soc Ophtal Fr 67:856–865

Campinchi R, Faure JP, Bloch-Michel E and Haut J (1970) L'uvéite. Phénomènes immunologiques et allergiques. Paris, Masson

Char DH (1978) Immunology of uveitis and ocular tumours. New York, Grune and Stratton

Clagett JA and Weigle WO (1974) Roles of T and B lymphocytes in the termination of unresponsiveness to autologous thyroglobulin in mice. J Exp Med 139:643–660

Cochrane CG (1969) Mediation of immunologic glomerular injury. Transport Proc 1:949–958

Cole DF (1974) The site of breakdown of the blood-aqueous barrier under the influence of vaso-dilator drugs. Exp Eye Res 19:591–607

Committee for HLA nomenclature, WHO, 1980

Cruchaud A, Chenais F, Fournié GJ, Humain L, Lambert PH, Mulli JC and Chatelant F (1975) Immune complex deposits in SLE kidney without histological or functional alteration. Eur J Clin Invest 5:297–309

Dausset J (1977) Le système HLA et les maladies. La Recherche 77:335–343

De Bruyère M (1978) Le système majeur d'histocompatibilité chez l'homme. Thèse, Université de Louvain

Dernouchamps JP, Vaerman JP, Michiels J and Heremans JF (1975) La transferrine des liquides endoculaires chez le lapin. Ophthalmologica 170:72–83

Dernouchamps JP and Michiels J (1976) La transferrine des liquides endoculaires chez l'homme et chez l'animal Acta XXII Concil Ophthalmol Paris, Masson Vol II, p. 199–205

Dernouchamps JP, Vaerman JP, Michiels J and Masson PL (1977) Immune complexes in the aqueous humor and serum. Am J Ophthalmol 84:24–31
Desmonts G (1966) Definitive serological diagnosis of ocular toxoplasmosis. Arch. Ophthalmology, 76:839–851
Dieckhues B (1965) Endogene Uveitis als Ausdruck eines bakteriell-allergischen Geschehens am Auge. Vortrag VI Europ Allerg Kongr Stockholm
Dieckhues B (1967) Allergie und Auge. Bücherei des Augenartzes, Heft 48. Enke Verlag Stuttgart
Diehl V, Jereb B, Stjermswärd J, O'Toole C and Ahström L (1971) Cellular immunity to nephroblastoma. Int J Cancer 7:277–284
Dive C (1970) Les protéines de la bile. Thèse Arscia, Bruxelles
Dive C and Heremans JF (1974) Nature and origin of the proteins of bile. I. A comparative analysis of serum and bile proteins in man. Eur J Clin Invest 4:235–239
Dive C, Nadalini RA, Vaerman JP and Heremans JF (1974) Origin and nature of the proteins of bile. II. A comparative analysis of serum, hepatic lymph and bile proteins in the dog. Eur J Clin Invest 4:241–246
Dixon FJ, Feldmann JD and Vazquez J (1961) Experimental glomerulonephritis. The pathogenesis of a laboratory model resembling the spectrum of human glomerulonephritis. J Exp Med 113:899–920
Doe WF, Booth CC and Browen DL (1973) Evidence for complement-binding immune complexes in adult coeliac disease, Crohn's disease and ulcerative colitis. Lancet 1:402–403
Duke-Elder S (1927) The nature of the intraocular fluids. Br J Ophthalmol suppl III
Eeckhout, Y, Riccomi H, Cambiaso C, Vaes G and Masson P (1976) Studies on properties common to collagen and C1q. Arch Int Physiol Biochim 84:611–612
Elschnig A (1910) Studien zur sympathischen Ophthalmie. II. Die Antigen Wirkung des Augenpigmentes. Graefes Arch Ophthal 76:509–546
Esser H, Heinzler F and Pau M (1954) Elektrophoretische Trennung der Eiweissfraktionen im menschlichen Kammerwasser. Graefes Arch Ophthalmol 155:11–16
Faure JP (1980) Autoimmunity and the retina. Curr Topics Eye Res 2:215–302
Faure JP, Blanck C and Offret G (1966) Analyse immuno-électrophorétique d'humeurs aqueuses inflammatoires. Arch Ophtalmologie 26:773–778
Faure JP, De Kozak Y, Dorey C and Torres R (1973) Production of retino-uveitis in guinea pigs by immunization with outer segments of heterologous retinal photoreceptor cells. Biomedicine 19:403–407
Fisher MM, Nagy B, Bazin H and Underdown BJ (1979) Biliary transport of IgA: role of secretory component. Proc Nat Acad Sci US 76:2008–2012
François J (1949) Etudes pupillaires dans l'hétérochromie de Fuchs. Bull Soc Belg Ophthal 91:154–165
François J (1961) Heredity in ophthalmology. St. Louis, Mosby, p 536
François J, Rabaey M and Evens L (1958) Agar microelectrophoresis of the aqueous humor. Arch Ophthalmology 59:692–702
François J and Rabaey M (1960) Microelectrophoresis on agar of normal and pathological aqueous humor. Arch Ophthalmology 63:836–849
Fuchs E (1906) Ueber Komplikationen der Heterochromie. Z Augenheilk 15:191–212
Gabay R, Zubler RH, Nydegger UE and Lambert PH (1977) Immune complexes and complement catabolism in ankylosing spondylitis. Arthr Rheum 20:913–916
Gamble CN, Wiesner KB, Shapiro RF and Boyer WJ (1979) Immune complex pathogenesis of glomerulonephritis and pulmonary vasculitis in Behçet's disease. Am J Med 66:1031–1039
Gelfand MC, Shin ML, Nagle RB, Green I and Frank MM (1976) The glomerular complement receptor in immunologically mediated renal glomerular injury. N Engl J Med 295:10–14
Georgiadès G (1956) Le syndrome 'hétérochromie-cataracte'. Bull Mém Soc Fr Ophtalmol 69:470–487
Germuth FG, Senterfit LB and Pollack AD (1967) Immune complexes disease. Experimental acute and chronic glomerulonephritis. Johns Hopkins Med J 120:225–241
Gitlin D, Kumate J, Urrusti J and Morales (1964) The selectivity of the human placenta

in the transfer of plasma proteins from mother to fetus. J Clin Invest 43:1938–1951
Gocke DJ, Morgan C, Lockshin M, Hsu K, Bombardieri S and Christian CL (1970) Association between polyarteriris and Australia antigen. Lancet IV:1149–1153
Goldmann H and Witmer R (1954) Antikörper im Kammerwasser. Ophthalmologica 127:323–330
Green FC and Feeney RE (1968) Physical evidence for transferrin as single polypeptide chains. Biochemistry 7:1366–1371
Grotte G (1956) Passage of dextran molecules across the blood-lymph barrier. Acta Chir Scand Suppl 211:1
Gupta RC, O'Duffy JD, McDuffie FC, Meurer M and Bordon RE (1978) Circulating immune complexes in active Behçet's disease. Clin Exp Immunol 34:213–218
Halbert SP, Locatcher-Khorazo D, Swick L, Witmer R, Seegal B and Fitzgerald P (1957) Homologous immunological studies of ocular lens. J Exp Med 105:439–462
Hedfors E and Norberg R (1974) Evidence for circulating immune complexes in sarcoidosis. Clin Exp Immunol 16:493–496
Heer G (1957) Il contenuto proteico dell'umor acqueo normale. Rass Ital Ottal 26:401–410
Hellström I, Hellström KE, Evans CA, Hepper GH, Pierce GE and Yang JPS (1969) Serum-mediated protection of neoplastic cells from inhibition by lymphocytes immune to their tumor-specific antigens. Proc Nat Acad Sci US 62:362–368
Hellström I, Sjögren HO, Warner G and Hellström KE (1971) Blocking of cell-mediated tumor immunity by sera from patients with growing neoplasms. Int J Cancer 7:226–237
Henry RM, Sobel C and Segelove M (1956) Turbidimetric determination of proteins with sulfosalicylic and trichloracetic acids. Proc Soc Exp Biol 92:748–751
Henson P (1971) The immunologic release of constituents from neutrophil leukocytes. I. The role of antibody and complement on nonphagocytosable surfaces or phagocytosable particles. J Immunol 107:1535–1546
Hinsberg K and Gleiss J (1950) Ueber die Bestimmung der Proteine im Liquor cerebrospinalis. Klin Wschr 28:444–448
Holmberg A (1959) The ultrastructure of the capillaries in the ciliary body. Arch Ophthalmology 62:949–951
Howes EL, Jr and McKay DG (1975) Circulating immune complexes. Effect on ocular vascular permeability in the rabbit. Arch Ophthalmology 93:365–370
Hurd ER, Lo Spalluto J, Burtonboy G, Kinsella TD and Ziff M (1971) Immune complexes as cellular inclusions. In: W. Müller, HG Harworth and K. Fehr (eds) Rheumatoid Arthritis. Pathogenetic Mechanisms and Consequences in Therapeutics (Colloquia Geigy). London, Academic Press, p 373
Ikui H, Mimatsu T, Maeda J and Tomita I (1960) Fine structure of the blood vessels in the iris. Kyushu J Med Sci 11:113–124
Israels ED, Nisli G, Paraskevas F and Israels LG (1973) Platelet Fc receptor as a mechanism for Ag-Ab complex induced platelet injury. Thrombos Diathes Haemorrh 29:434–444
Izui S, Zaldivar NM, Scher I and Lambert PH (1977) Mechanisms for induction of anti-DNA antibodies by bacterial lipopolysaccharides in mice. I. Anti-DNA induction by LPS without significant release of DNA in circulating blood. J Immunol 119: 2151–2156
Izui S, Eisenberg RA and Dixon FJ (1979) IgM rheumatoid factors in mice injected with bacterial lipopolysaccharides. J Immunol 122:2096–2102
Jackson GDF, Lemaître-Coelho I, Vaerman JP, Bazin H and Beckers A (1978) Rapid disappearance from serum of intravenously injected rat myeloma IgA and its secretion into bile. Eur J Immunol 8:123–126
Jamieson GA (1965) Studies on glycoproteins. II. Isolation of the carbohydrate chains of human transferrin. J Biol Chem 240:2914–2920
Jewel DP and MacLennan ICM (1973) Circulating immune complexes in inflammatory bowel disease. Clin Exp Immunol 14:219–226
Jose DG and Seshadri R (1974) Circulating immune complexes in human neuroblastoma: direct assay and role in blocking specific cellular immunity. Int J Cancer 13: 824–838

Kilburn DG, Fairhurst M, Levy JG and Whitney RB (1976) Synergism between immune complexes and serum from tumor-bearing mice in the suppression of mitogen responses. J Immunol 117:1612–1617
Krause U and Raunio V (1970) The proteins of the pathologic human aqueous humour. An in vivo investigation. Ophthalmologica 160:280–287
Kronfeld PC (1941) The protein content of the aqueous humor in man. Am J Ophthalmol 24:1121–1131
Kunkel HG, Franklin EC and Müller-Eberhard HJ (1959) Studies on the isolation and characterization of the 'rheumatoid factor'. J Clin Invest 38:424–434
Laffers Z and Bozsoky S (1959) Die klinische Bedeutung der Antistreptolysinbestimmung im Kammerwasser. Ophthalmologica 137:322–334
Landis EM and Pappenheimer JR (1963) Handbook of Physiology. II. Circulation. Amer Physiol Soc, Washington, p 961–1021
Laterre EC (1965) Les protéines du liquide céphalo-rachidien à l'état normal et pathologique. Thèse Arscia, Bruxelles
Laurell CB (1965) Antigen-antibody crossed electrophoresis. Anal Biochem 10:358–361
Luntz MH (1964) Auto-immune response to lens and uveal protein. S Afr Med J 3:130–133
Luntz MH (1968) Anti-uveal and anti-lens antibodies in uveitis and their significance. Exp Eye Res 7:561–569
Lurhuma AZ, Cambiaso CL, Masson PL and Heremans JF (1976) Detection of circulating antigen-antibody complexes by their inhibitory effect on the agglutination of IgG-coated particles by rheumatoid factor or C1q. Clin Exp Immunol 25:212–226
Lurhuma AZ, Riccomi H and Masson PL (1977) The occurrence of circulating immune complexes and viral antigens in idiopathic thrombocytopenic purpura. Clin Exp Immunol 28:49–56
Maisel H (1963) The immunologic specificity of lens antigen. Am J Ophthalmol 55: 1208–1216
Makley TA Jr (1956) Heterochromic cyclitis in identical twins. Am J Ophthalmol 41: 768–772
Mancini G, Carbonara AO and Heremans JF (1965) Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 2:235–254
Mann KG, Fish WW, Cox AC et al (1970) Single chain nature of human serum transferrin. Biochemistry 9:1348–1354
Manuel Y (1969) Etude comparative de la composition protéique du liquide sous-rétinien et de la composition protéique des autres liquides oculaires. Bull Soc Ophtalmol Fr 69:623–625
Mapstone R and Woodrow JC (1975) HLA-B27 and acute anterior uveitis. Br J Ophthalmol 59:270–275
Marak GE, Aye MS and Alepa FP (1973) Cellular hypersenstitivity in penetrating eye injuries. Invest Ophthalmol 12:380
Marak GE, Font RL and Weigle WO (1979) Pathogenesis of lens-induced endophthalmitis. In: Silverstein and O'Connor (eds) Immunology and Immunopathology of the Eye. New York, Masson, p 135–137
Marder RJ, Rent R, Choi EYC and Gewurz H (1976) C1q deficiency associated with urticarial-like lesions and cutaneous vasculitis. Am J Med 61:560–565
Marney SR and Des Prez RM (1971) Rabbit platelet injury by soluble antigen and antibody. 1. Inhibitory action of citrate, TAME and ACTEE. J Immunol 106:74–81
Martenet AC (1970a) Anticorps viraux dans l'uvéite. Ophthalmologica 161:243–244
Martenet AC (1970b) Anticorps contre l'uvée. Bull Mém Soc Fr Ophtalmol 83:97–101
Mayerson HS, Wolfram CG, Shirley HH Jr and Wasserman K (1960) Regional differences in capillary permeability. Am J Physiol 198:155–160
McPherson SD and Woods AC (1948) The significance of the intracutaneous test for hypersensitivity to uveal pigment. Am J Ophthalmol 31:35–45
Meulemans O (1960) Determination of total protein in spinal fluid with sulphosalicylic acid and trichloracetic acid. Clin Chim Acta 5:757–761
Meyers RL, Shabo AL and Maxwell DS (1975) Effect of prostaglandin on the blood-aqueous barrier in the rabbit ciliary process. Prostaglandins 9:167

Michiels J (1968) L'humeur aqueuse et la barrière hémato-camérulaire. Thèse. Bruxelles, de Visscher
Michiels J and Dernouchamps JP (1971) L'hétérochromie de l'iris. Bull Mém Soc Fr Ophtalmol 84:395–411
Michiels J and Dernouchamps JP (1976) Quantitation of specific antibodies in the aqueous humor. Mod Probl Ophthalmol 16:131–136
Miescher P and Cooper N (1960) The fixation of soluble antigen-antibody complexes upon thrombocytes. Vox Sang 5:138
Missotten L (1964) L'ultrastructure des tissus oculaires. Bull Soc Belg Ophtalmol 136
Morgan EH (1968) Plasma protein turnover and transmission to the milk in the rat. Biochim Biophys Acta 154:478–487
Morgan EH (1969) Factor affecting the synthesis of transferrin by rat tissue slices. J Biol Chem 244:4193–4199
Morris AD, Littleton C, Corman LC Esterly J and Sharp GC (1975) Extractable nuclear antigen effect on the DNA anti-DNA reaction and NZB/NZW mouse nephritis. J Clin Invest 55:903–907
Müller-Eberhard HJ (1975) Complement. Ann Rev Biochim 44:697–794
Natvig JB, Munthe E, Mellbye OJ and Kars E (1974) Studies on the immunopathology of rheumatoid inflammatory joint tissue. Acta Pathol Microbiol Scand Sect A, Suppl 248:145–151
Neufeld AH and Sears ML (1973) The site of action of prostaglandin E_2 on the disruption of the blood-aqueous barrier of the rabbit eye. Exp Eye Res 17:445
Neupert JR and Lawrence C (1970) Protein release during aqueous withdrawal in rabbits. Invest Ophthalmol 9:865–872
Nies KM, Brown JC, Dubois EL, Quismorio FP, Friou GJ and Terasaki PI (1974) Histocompatibility (HLA) antigens and lymphocytotoxic antibodies in systematic lupus erythematosus (SLE). Arthritis Rheum 17:397–402
Norberg R (1974) IgG complexes in serum of rheumatoid arthritis patients. Scand J Immunol 3:229–236
O'Brien IG and Gibson F (1970) The structure of enterochelin and related 2, 3-dihydroxy-N-benzoyl-serine conjugates from E coli. Biochim Biophys Acta 215:393–402
Oeff K (1959) In: H. Peeters (ed) Protides of the Biological Fluids. Proceedings of the 7th Colloquium of Bruges. Elsevier, Amsterdam p 164–170
Offret G, Saraux H and Bisson J (1955) Résultats de deux années d'étude de l'humeur aqueuse. Bull Mém Soc Fr Ophtalmol 68:51–57
Ohno S, Sugiura S, Ohguchi M and Aoki K (1979) Close association of HLA-B5 with Behçet's disease In: Silverstein and O'Connor (eds) Immunology and Immunopathology of the Eye. Masson, New York, p 15–17
Orlans E, Peppard J, Reynolds J and Hall J (1978) Rapid active transport of immunoglobulin A from blood to bile. J Exp Med 147:588–592
Osler AG and Siraganian RP (1972) Immunologic mechanisms of platelet damage. Progr Allergy 16:450–498
Ouchterlony O (1949) Antigen-antibody reactions in gels. Acta Pathol Microbiol Scand 26:507–515
Palade GE, Simionescu M and Simionescu N (1978) Transport of solutes across the vascular endothelium. In: SC Silverstein (ed) Transport of macromolecules in cellular systems. Dahlem Konferenzen, Berlin, p 145–166
Palmour RM and Sutton HE (1971) Vertebrate transferrins, molecular weights, chemical compositions, and iron-binding studies. Biochemistry 10:4026–4032
Pappas GD and Tennyson VM (1962) An electron microscopic study of the passage of colloidal particles from the blood vessels of the ciliary processes and choroid plexus of the rabbit. J Cell Biol 15:227–239
Pappenheimer JR (1953) Passage of molecules through capillary walls. Physiol Rev 33:387–423
Parker WC and Bearn AG (1962) Studies on the transferrins of adult serum, cord serum and cerebrospinal fluid. The effect of neuraminidase. J Exp Med 115:83–105
Penttinen K, Vaheri A and Myllylä G (1971) Detection and characterization of immune complexes by the platelet aggregation test. I. Complexes formed in vitro. Clin Exp Immunol 8:389–397

Peretz WL and Tomasi TB (1961) Aqueous humor proteins in uveitis. Arch Opthalmology 65:20–23
Perkins ES (1961) Uveitis and toxoplasmosis. London, Churchill
Perkins ES (1968) Uveitis survey at the Institute of Opthalmology, London. In: SB Aronson (ed) Clinical Methods in Uveitis. Saint Louis, Mosby, p 58–65
Perkins ES and Wood RM (1964) Auto-immunity in uveitis. Br J Ophthalmol 48:61–69
Perrin LH, Lambert PH and Miescher PA (1975) Complement breakdown products in plasma from patients with systemic lupus erythematosus and patients with membrano-proliferative or other glomerulonephritis. J Clin Invest 56:165–176
Pincus T, Haberkern R and Christian CL (1968) Experimental chronic glomerulitis. J Exp Med 127:819–831
Pirquet C (von) (1906) Allergie. Münch Med Wschr 53:1457
Pollack JR and Neilands JB (1970) Enterobactin, an iron transport compound from Salmonella typhrimurium. Biochem Biophys Res Comm 38:989–992
Pope RM, Teller DC and Mannik M (1974) The molecular basis of self-association of antibodies to IgG (rheumatoid factors) in rheumatoid arthritis. Proc Nat Acad Sci USA 71:517–521
Priluck IA, Robertson DM, Pritchard DJ and Ilstrup DM (1979) Immune responsiveness in patients with choroidal malignant melanoma. Am J Ophthalmol 87:215–220
Raviola G (1977) The structural basis of the blood-ocular barriers. Exp Eye Res Suppl 27–63
Remky H (1954) Klinische Methoden zur quantitativen und qualitativen Untersuchung von Kammerwasser-Protein Graefes Arch Ophthalmol 156:85–89
Remky H (1958) Zur Klinik der serologisch diagnostizierten Augentuberkulose. Klin Monatsbl Augenheilkd 133:518–529
Remky H (1960) L'uvéite tuberculeuse. Diagnostic sérologique, formes cliniques et thérapie spécifique. Bull Mém Soc Fr Ophtalmol 73:64–79
Remky H (1965a) Etiological diagnosis of endogenous uveitis. Int Ophthalmol Clin 5:789–822
Remky H (1965b) Auto-anticorps, facteurs iridogènes de l'iritis? Bull Mém Soc Fr Ophtalmol 78:401–409
Rogers HJ (1967) Bacteriostatic effects of horse sera and serum fraction on Clostridium welchii type A, and the abolition of bacteriostasis by iron salts. Immunology 12: 285–301
Rogers HJ (1975) Bacterial iron metabolism and host resistance. In: D. Schlessinger (ed) Microbiology. Am Soc for Microbiol, Washington, p 289–298
Rosen A, Gergely P, Jondal M and Klein G (1977) Polyclonal Ig production after Epstein-Barr virus infection of human lymphocytes in vitro. Nature 267:52–54
Rosenthal M, Graf U and Müller W (1976) Nachweis und Vorkommen von Immunkomplexen bei rheumatischen Erkrankungen. Schweiz Med Wschr 106:1116–1122
Russell ML, Gordon DA and Broder I (1974) Studies into the occurrence of soluble antigen-antibody complexes in disease. VII. Clinical laboratory correlations of immunoglobulin complexes in rheumatoid synovial fluid. J Rheumatol 1:153
Sallmann L von and Moore DH (1948) Electrophoretic patterns of concentrated aqueous humor of rabbit, cattle and horse. Arch Opthalmology 40:279–284
Sandberg HO, Folling I and Closs O (1974) Quantitation of alpha-crystallin in human aqueous humor by radioimmunoassay. Acta Ophthalmol 52:161–170
Scharmann W, Brückler J and Blobel H (1971) Wirkung bakterieller Neuraminidasen auf Transferrin von Menschen, Rind und Kaninchen. Biochim Biophys Acta 229: 136–142
Schlaegel TF Arbogast JL and Estela LA (1961) The value of the Middlebrook-Dubos hemagglutination test for tuberculosis. Am J Ophthalmol 51:627–634
Schlosstein L, Terasaki PI, Bluestone R and Pearson CM (1973) High association of an HL-A antigen, W27, with ankylosing spondylitis. N Engl J Med 288:704–706
Schultze HE and Heremans JF (1966) Molecular Biology of human Proteins. I. – Nature and Metabolism of extracellular Proteins. Elsevier, Amsterdam
Sedan J and Guillot P (1955) Uvéites médicamenteuses. Bull Mém Soc Fr Ophthalmol 68:317–323

Sergent JS, Lockshin MD, Christian CL and Gocke DG (1976) Vasculitis with hepatitis B antigenaemia. Medicine 55:1–18

Sery TW and Huang L (1968) Diagnostic procedure to determine autoimmunity to basement membranes antigens. In: SB Aronson (ed) Clinical Methods in Uveitis. Saint Louis, Mosby, p 144

Shabo AL and Maxwell DS (1972) The blood-aqueous barrier to tracer protein: a light and electron microscopic study of the primate ciliary process. Microvasc Res 4: 142–158

Shakib M and Cunha-Vaz JG (1966) Studies on the permeability of the blood-retinal barrier. IV. Functional complexes of the retinal vessels and their role in the permeability of the blood-retinal barrier. Exp Eye Res 5:229

Simionescu N, Simionescu M and Palade GE (1975) Permeability of muscle capillaries to small hemepeptides. Evidence for the existence of patient transendothelial channels. J Cell Biol 64:586–607

Soltis RD, Hasz D, Morris MJ and Wilson ID (1979) Evidence against the presence of circulating immune complexes in chronic inflammatory bowel disease. Gastroenterology 76:1380–1385

Soltys HD and Brody JI (1970) Synthesis of transferrin by human peripheral blood lymphocytes. J Lab Clin Med 75:250–257

Stachelin LA (1974) Structure and function of intercellular junctions. Int Rev Cytol 39:191–283

Steiger R, Böhringer H and Wunderly C (1955) Quantitative und elektrophoretische Untersuchungen am neugebildeten Kammerwasser normaler menschlicher Augen. Klin Monatsbl Augenheilkd 126:490–492

Stjernschantz J, Uusitalo R and Palkama A (1973) The aqueous proteins of the rat in normal eye and after aqueous withdrawal. Exp Eye Res 16:215–221

Tagawa Y and Sugiura S (1978) HLA and Vogt-Koyanagi-Harada disease. Acta XXIII Concil Ophthal Kyoto II:944–946

Taniguchi Y (1962) Fine structure of blood vessels in the ciliary body. Jap J Ophthalmol 6:93–103

Theodore FH and Lewson AC (1939) Bilateral iritis complicating serum sickness. Arch Ophthalmology 21:828–832

Thorbecke GJ, Liem HH, Knight S, Cox K and Müller-Eberhard U (1973) Sites of formation of the serum proteins transferrin and hemopexin. J Clin Invest 52:725–731

Tousimis A and Fine BS (1959) Ultrastructure of the iris. Am J Ophthalmol 48:307–401

Trepo C and Thivolet J (1970) Antigène Australia, hépatite virale et périartérite noueuse. Presse Méd 78:1575

Tulsiani DRP, Nordquist RE and Carubelli R (1973) The neuraminidases of rat eyes. Exp Eye Res 15:93–103

Unger WG, Cole DF and Hammond B (1975) Disruption of the blood-aqueous barrier following paracentesis in the rabbit. Exp Eye Res 20:255–270

Van Snick JL, Van Roost E, Markowetz B, Cambiaso CL and Masson PL (1978) Enhancement by IgM rheumatoid factor of in vitro ingestion by macrophages and in vivo clearance of aggregated IgG or antigen-antibody complexes. Eur J Immunol 8:279–285

Vegge T (1971) An epithelial blood-aqueous barrier to horseradish peroxidase in the ciliary processes of the vervet monkey (Cercopithecus aethiops). Z Zellforsch 114: 309–320

Vegge T, Neufeld AH and Sears ML (1975) Morphology of the breakdown of the blood-aqueous barrier in the ciliary processes of the rabbit eye after prostaglandin E2. Invest Ophthalmol 14:33

Verrey F (1954) Clinique de l'humeur aqueuse pathologique. Delachaux et Niestlé Neuchâtel

Verrey F (1974) Personal communication

Verrier-Jones J, Cumming RH, Bucknall RC, Asplin CM, Fraser ID, Davis P and Hamblin TJ (1976) Plasmapheresis in the management of acute systemic lupus erythematosus. Lancet I:709–711

Vinceneux P (1977) Association urticaire, vascularite et déficit en C1q. Nouv Presse Méd 6:234
Vogt A (1942) Lehrbuch und Atlas der Spaltlampenmikroskopie des lebenden Auges. Schweizer Druck und Verlagshaus, Zurich, Vol III, p 886
Wacker WB and Lipton MM (1965) Experimental allergic uveitis: homologous retina as uveitogenic antigen. Nature 206:253–254
Whitelocke RAF, Eakins KE and Bennett A (1973) Acute anterior uveitis and prostaglandins. Proc Roy Soc Med 66:429–434
Williams BD and Lehner T (1977) Immune complexes in Behçet's syndrome and recurrent oral ulceration. Brit Med J 1:1387–1389
Williams J (1968) A comparison of glycopeptides from the ovotransferrin and serum transferrin of the hen. Biochem J 108:57–67
Williams J (1974) The formation of iron-binding fragments of hen ovotransferrin by limited proteolysis. Biochem J 141:745–752
Williams RC Jr, and Kunkel HG (1963) Antibodies to rabbit gamma-globulin after immunizing with various preparations of autologous gamma-globulins. Proc Soc Exp Biol Med 112:554–561
Winchester RJ, Agnello V and Kunkel HG (1970) Gamma-globulin complexes in synovial fluids of patients with rheumatoid arthritis. Partial characterization and relationship to lowered complement levels. Clin Exp Immunol 6:689–706
Winfield JB, Kooler D and Kunkel HG (1975) Role of DNA-anti-DNA complexes in the immunopathogenesis of tissue injury in systemic lupus erythematosus. Scand J Rheumatol 11:59–64
Wirostko E and Spalter HF (1967) Lens-induced uveitis. Arch Ophthalmology 78:1–7
Witmer R (1954) Periodic ophthalmia in horses. Am J Ophthalmol 37:243–253
Witmer R (1955a) Antibody formation in the rabbit eye studied with fluorescein-labelled antibodies. Arch Ophthalmology 53:811–816
Witmer R (1955b) Ätiologische Diagnostik der Uveitis. II. Mitteilung. Die Hämagglutination nach Middlebrook und Dubos und die Papier-elektrophorese der experimentellen Kaninchentuberkulose. Graefes Arch Ophthalmol 156:337–355
Witmer R (1962) Serologie der Uveitis. Ophthalmologica 143:357–362
Witmer R (1967) The significance of ocular antibodies in endogenous inflammatory disease of the eye. Mod Trends Ophthalmol 4:117–129
Witmer R (1968) Uveitis survey at the University Eye Clinic in Zurich. In: SB Aronson (ed) Clinical Methods in Uveitis. Saint Louis, Mosby, 1968 p 48–57
Witmer R (1976) History of immunology of uveitis. Mod Probl Ophthalmol 16:8–11
Witmer R (1979) Immunology of the aqueous humor. Acta XXIII Concil Ophthalmol Kyoto 1978, Excerpta Medica, Amsterdam, p 111–116
Witmer R and Martenet AC (1964) Experimentelle phakoantigene Uveitis am Kaninchen. Ber dtsch Ophthal Ges 66:59–61
Woods AC (1921) Immune reactions following injuries to the uveal tract. JAMA 77: 1312
Wunderly C and Cagianut B (1952) Electrophorèse de l'humeur aqueuse pathologique. Ann Ocul 185:414–421
Wunderly C, Steiger R and Böhringer HR (1954) Neue mehrfache Untersuchungen am gleichen Kammerwasser menschlicher Augen. Experientia 10:432–433
Yanagizawa T (1965) Protein fractions of aqueous humour. Acta Soc Ophthalmol Jap 69:2015–2021
Zoller B, Arnold W, Hutteroth TH and Meyerzumbuschenfelde KH (1978) Determination of immune complexes of various origins in periarteritis nodosa. Z Rheumatol 37:142
Zondag HA and Van Boetselaer GL (1960) Determination of protein in cerebrospinal fluid. Sources of error in the Lowry method. Clin Chim Acta 5:155–156