Clinical, biochemical, and immunoelectrophoretic study of subretinal fluid

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Subretinal fluid found in retinal detachments associated with retinal tears is derived from the vitreous, or from the choroidal vessels, or both. The relatively high concentration of protein found in subretinal fluid argues mainly in favour of the protein being derived from the choroidal vessels. Examinations of total protein concentration in subretinal fluid have been made by several workers. Smith and Douty (1960) and Heath, Beck, and Foulds (1962) found that the total protein concentration of the subretinal fluid tended to rise with an increase in the duration of the detachment. Heath & others (1962) felt that the rise in total protein concentration indicated an increasing permeability of the choroidal capillaries; they also found a marked increase in total protein in eyes which had undergone more than one operation. Weber and Wilson (1963) confirmed the protein findings and later stated (Weber and Wilson, 1966) that they thought the formation of subretinal fluid was a dynamic process. Cooper, Halbert, and Manski (1963), using immunoelectrophoresis, showed large numbers of serum proteins in the fifteen cases of subretinal fluid examined. Manuel, Royer, Richard, and Creyssel (1960), examining subretinal fluid by immunoelectrophoresis, found a "γ" precipitin band. Speiser (1964) used a similar technique in examining eleven cases and found serum-like proteins when the total protein concentration was high, but with lower protein concentrations the globulins were relatively reduced, and with very low total protein concentrations only albumin could be detected.

The purpose of this communication is to report the total protein concentrations and immunoelectrophoretic examinations of 28 specimens of subretinal fluid obtained from 26 patients suffering from retinal detachment. The technique of immunoelectrophoresis used in this study has enabled a more detailed examination of the subretinal fluid to be made than has hitherto been reported. It has allowed in particular a detailed examination of the γ-globulin fraction of the subretinal fluid. The presence or absence of IgG, IgA, and IgM may be detected, and thus it is possible to assess the degree of permeability of the choroidal capillaries and Bruch's membrane in retinal detachments.

Method

COLLECTION AT OPERATION

The area around the proposed site of scleral puncture was carefully dried. Scleral puncture was

made immediately after the application of cryotherapy. Subretinal fluid was collected by placing dried, sterilized, 20- μ l. glass capillary tubing in the stream of subretinal fluid emerging from the puncture site. These tubes were then sealed and stored at 4°C. and analysed as soon as possible.

ANALYSIS

Total protein

This was measured by micro-Kjeldahl digestion followed by nitrogen estimation with Nessler's reagent. To enable the protein electrophoretic pattern of the subretinal fluid to be compared with that of plasma from the same patient, a drop of fluid was concentrated by rapid evaporation in a stream of warm air. The concentrated fluid was then placed in one "foot" of the multiapplicator, the patient's plasma was placed in an adjacent foot, and cellulose acetate electrophoresis was performed with Ponceau S staining (Carruthers, 1970). The identical conditions for migration and staining enabled the subretinal fluid and plasma patterns to be correlated, both by inspection and by scanning on a Wooster Mark III Microdensitometer. The results obtained were expressed in g./100 ml.

IMMUNOELECTROPHORESIS

This was performed on agar plates based on the microtechnique proposed by Scheidegger (1955) as an improvement on the original technique of Grabar and Williams (1953). 8 ml. of 1.5 per cent. I.D. agar (oxoid) in veronal buffer (pH 8.2; $I = 0.05$) to which sodium azide was added to give a concentration of 1 : 1000 was poured over a previously coated 8 $\times$ 8 cu.cm. glass slide (Kodak) and allowed to set in a moist chamber overnight. Circular wells 1 mm. in diameter were cut along the middle of the plate 10 mm. apart and filled with subretinal fluid using a micropipette. Normal human serum was put into one of the wells for comparison. The plate was then put upside down on the shoulder piece of the electrophoresis tank (Shandon U77 after Kohn) and connected to electrode compartments containing barbitone buffer (pH 8.6; $I = 0.075$) by thick filter paper strips. The current was adjusted to 10 mA/per plate and allowed to run for 2 hrs. After electrophoresis the plate was removed and troughs (60 $\times$ 1 mm.) were cut equidistant between the wells, along the direction of electrophoretic migration. Strips of agar were removed by applying suction. The troughs were then filled with antihuman multivalent and monospecific sera and the plate was then left in a moist chamber for 2 days. The plate was then washed with 3 per cent. saline for 2 days to remove unprecipitated proteins. After further washing in distilled water for 4 hrs the plate was covered by lint-free filter paper and completely dried in an incubator at 37°C. After removal of the filter paper the plate was stained in 0.2 per cent. Ponceau S or 0.1 per cent. nigrosine to pick up fine precipitin lines, otherwise barely visible. According to the nature and number of precipitin lines, as compared with normal human serum the subretinal fluids were divided into three main groups:

Group A Possessed almost all the constituents of normal serum and showed prominent precipitin bands for albumin, α_1 antitrypsin, group-specific component (Gc)-globulin, haptoglobin, transferrin, haemopexin, IgG and IgA.

Group B Possessed all the constituents shown in Group A, with the exception of IgA which was absent.

Group C Possessed few of the constituents of normal human serum. Only albumin, traces of IgG, and transferrin were found.

Three cases showed the characteristics of Group B, but minute traces of IgA were found and they were indicated as belonging to Group A-B. Examples of the results obtained from immunoelectrophoresis are shown in Figs 1-4 (opposite and overleaf).

FIG. 1 Sample of subretinal fluid tested against antihuman "whole" or multivalent serum (WS) and mono-specific sera against immunoglobulins G, A, and M. This sample would be classified under Group A as only IgM is absent

FIG. 2 Subretinal fluid of Group B pattern, where IgA as well as IgM is absent

FIG. 3 Subretinal fluid of Group C pattern, where albumin and only traces of IgG and transferrin are present

FIG. 4 Group of six subretinal fluids, of which according to the classification described above, Nos 2, 3, 4, and 6 are Group B. No 1, showing only albumin and No. 5, showing all the fractions indicated in Fig. 3, are both Group C.

Results (Table I, opposite)

The duration of the retinal detachment is indicated in weeks. Total protein is expressed in g./100 ml. Specific points of clinical interest are shown. Unless indicated, subretinal fluid specimens were obtained from patients undergoing an initial retinal detachment operation and retinal holes were found. One case of retinoschisis was studied.

EXAMINATION OF THE RESULTS SHOWS THE FOLLOWING:

(1) High levels of total protein concentration (71.5 g./100 ml.) are found in cases of long-standing retinal detachment (longer than 8 weeks), as shown in Table II.

Table II Protein concentration of subretinal fluid related to duration of detachment

Duration of detachment (wks)	Total protein concentration (g./100 ml.)					
	< 1.0		1-1.50		> 1.50	
	No.	Per cent.	No.	Per cent.	No.	Per cent.
1-4 (13 cases)	7	53.8	6	46.2	0	0
8+ (12 cases)	6	50	2	16.7	4	33.3

(2) In the immunoelectrophoretic study there was a general tendency for subretinal fluid to come to resemble serum more closely, with increasing duration of detachment (i.e. towards Group A with increasing age), as shown in Table III (overleaf).

(3) There does not appear to be a constant relationship between immunoelectrophoresis grouping and total protein concentration. High total protein concentration may be associated with immunoelectrophoretic Group A (Cases 21, 24, and 26) or Group C

Table I Results in 28 specimens obtained from 26 patients

Case no.	Duration of detachment (wks)	Total protein (g/100 ml.)	Immuno-electrophoretic group	Details of detachment
1	1	0.94	C	Detachment operation 3 months previously
2	1	0.99	A→B	Aphakia
3	1	0.91	A	
4	1	0.69	C	
5	1	0.66	B	
6	1	1.45	C	
7	2	1.20	B	
8	2	1.24	C	
9	4	0.39	A	
10	4	1.22	A	Re-operation on Case 9
11	4	1.17	A	Aphakia
12	4	1.0	B	
13	4	Not done	C	
14	4	0.57	A→B	Aphakia
15	4	Not done	B	
16	8	0.79	A→B	Diathermy 5 yrs previously
17	8	0.92	B	
18	8	0.56	A	
19	8	0.84	A	Re-operation on Case 6
20	12	2.50	B	
21	12	1.70	A	
22	12	1.08	A	Diabetic traction detachment No holes
23	14	0.75	A	Diabetic traction detachment Holes seen
24	14	1.72	A	No holes seen Dense lens opacities
25	20	0.94	B	Retinal dialysis
26	24	2.44	A	No holes seen Congenital cataracts
27	60	1.37	C	Retinoschisis
28	64	1.13	B	

Table III *Duration of detachment related to immunoelectrophoretic properties of subretinal fluid*

Duration of detachment (wks)	Immunoelectrophoretic group							
	A		A-B		B		C	
	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.
1-4 (15 cases)	4	26.7	2	13.3	4	26.7	5	33.3
8+ (12 cases)	7	58.3	1	8.3	4	33.3	0	0

(Cases 6 and 8). Similarly low total protein concentration may be associated with immunoelectrophoretic Group A (e.g. Cases 3, 9, 18, 19, and 23) or Group C (Cases 1 and 4).

(4) Two patients in the series were studied twice. Case 9 initially showed a low total protein concentration (0.39 g./100 ml.), but this had increased (1.22 g./100 ml.) when a second operation was performed 3 months later (Case 10); the immunoelectrophoretic group (Group A) was unchanged. Case 6 was operated on again 3 months later (Case 19), and whereas the total protein concentration had decreased from 1.45 to 0.84 g./100 ml. the immunoelectrophoretic grouping had changed from C to A.

(5) Case 27 showed retinoschisis. The total protein concentration was 1.13 g./100 ml. and few of the normal serum constituents were present on immunoelectrophoresis (Group C).

(6) Cases 22 and 23 showed diabetic detachments. Case 22 was a typical traction detachment and Case 23 had two small retinal holes in addition to the traction appearance. In both the subretinal fluid resembled normal serum (Group A).

(7) Cases 1, 10, and 19 were re-operations. The total protein concentration was not notably raised. In Case 1 (Group C) the retina had become flat with absorption of subretinal fluid after the previous operation and had become re-detached at a later date. In Cases 10 (Group A) and 19 (Group A) the subretinal fluid had not absorbed after the initial operation.

Discussion

The technique of immunoelectrophoresis used in this study shows that subretinal fluid tends to come to resemble normal serum more closely as the duration of the retinal detachment increases. IgM (M.wt 900,000) was not detected in any of the cases studied. Table IV shows the molecular weight of proteins found in subretinal fluid and their concentrations in normal serum as described by Dettelbach and Ritzman (1968) and Rowe (1970). The findings of the immunoelectrophoretic study suggests that the chorio-capillaris and Bruch's membrane vary in their permeability. The change in the nature of subretinal fluid as the duration of the detachment increases may be explained by an increasing permeability of the chorio-capillaris, as suggested by Heath and others (1926). The absence in all cases of IgM, which has a high molecular weight, indicates that the permeability of the capillary endothelium and of Bruch's membrane is limited even in long-standing and re-operated cases, thus in no case did the subretinal fluid have protein characteristics of a true exudate. A constant relationship between immunoelectrophoretic

examination and total protein concentration was not found and it is felt that immunoelectrophoretic examination is a more reliable indicator of dysfunction of the chorio-capillaris and Bruch's membrane than total protein concentration.

Table IV *Molecular weight of proteins in subretinal fluid and their concentrations in serum*

<i>Proteins</i>	<i>Molecular weight</i>	<i>Concentration in serum (mg./100 ml.)</i>
Albumin	69,000	3,500-4,500
α_1 Antitrypsin	45,000	210- 500
Gc-globulin	50,800	40- 75
Haptoglobin	100,000	30- 190
Haemopexin	80,000	80- 100
Transferrin	90,000	230- 320
IgA	186,000	156- 294
IgG	150,000	853-1,463

Summary

In 28 specimens of subretinal fluid obtained from 26 patients the following observations were made:

- (1) High total protein concentration levels are found in long-standing retinal detachments. This confirms the work of others.
- (2) The technique of immunoelectrophoresis used in this study enabled a more detailed examination of subretinal fluid to be made than has previously been reported. Subretinal fluid comes to resemble normal serum more closely with increasing duration of the detachment. IgG and IgA were detected but IgM was absent. The findings support the theory of a limited semipermeability of the endothelium of the chorio-capillaris and of Bruch's membrane in patients with retinal detachments.
- (3) There is no apparent relationship between immunoelectrophoretic findings and total protein concentration estimations. It is suggested that immunoelectrophoretic examinations offer a more reliable indicator of chorio-capillaris dysfunction than do total protein concentration estimations.

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