

Viscosity and retinal vein thrombosis

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Retinal vein occlusions (RVO) have been well documented since Leber (1877) and Michel (1878) first described branch and central retinal vein occlusions respectively. However, no further progress was made until Novotny and Alvis (1961) introduced fluorescein angiography, which demonstrated the phenomenon of capillary non-perfusion, found in some cases of RVO, and gave investigators a useful tool for trying to determine the pathophysiology of the condition. A direct role for arterial occlusion in producing the clinical features of RVO has been discounted (Kohner and Shilling, 1976), but arterial disease is generally agreed to have a significant role in the aetiology. Although a much debated point, a complete thrombotic occlusion of the vein is generally thought not to occur. The pathological process at the site of the occlusion consists of changes in the vessel walls, blood constituents, and blood flow. These three components of Virchow's triad are interrelated, and changes in the vessel wall and blood flow have been investigated in man and animals by techniques of pathology (Rabinowicz, Litman, and Michaelson, 1968) and by angiography (Kohner, Dollery, Shakib, Henkind, Paterson, de Oliveira, and Bulpitt, 1970; Fujino, Curtin, and Norton, 1968). The importance of the effect of the blood constituents on the blood viscosity has received little attention, although Begg (1969) suggested this as a useful area of investigation.

Viscosity may be defined as the friction between two moving planes of fluid. Mathematically it may be defined as the ratio of the applied force (shear stress) to the differential velocity of the two planes (shear rate). For simple fluids the rate of flow is directly proportional to the applied force (Newtonian behaviour). Blood, however, does not show this simple relationship and is termed non-Newtonian. Therefore when measuring whole-blood viscosity, as in the present study, a wide

range of shear rates must be used in order to include all those which might be met in a normal or pathological circulation. Cone-plate and coaxial cylindrical viscometers have been widely used in this and other studies of whole-blood viscosity because the shear rate can be accurately monitored. The viscosity of plasma, which may be considered to behave in a Newtonian fashion (Dintenfass, 1971), may for this reason be accurately measured in a capillary viscometer in which the shear rates are inconstant, and this technique has also been used in the present investigation. Yield stress of a material under shear is the applied force necessary to overcome frictional forces between the sliding planes and so induce movement (Whitmore 1968). Blood has been shown to exhibit this rheological property (Merrill, Margetts, Cokelet, and Gilliland, 1965) and therefore the role of yield stress was also investigated.

Increased blood viscosity is associated with various pathological conditions in which vascular thrombosis or occlusion is a feature. Suggested associations are postoperative deep vein thrombosis (Dormandy and Edelman, 1973), recent cerebral infarction (Ott, Lechner, and Aranibar, 1974), acute myocardial infarction (Burch and De Pasquale, 1963), hypertension (Tibblin, Bergentz, Bjure, and Wilhelmsen, 1965), angina (Dormandy, 1975), and diabetes (Ditzel, 1967). The role of blood viscosity in retinal vein occlusion seems to have received little attention apart from the restricted studies of McGrath, Penny, Wechsler, and Hunyor (1973). The present study was designed to examine the role of viscosity and blood flow in the aetiology of retinal vein occlusion and the development of capillary non-perfusion.

Patients and methods

Forty-four unselected patients with retinal vein occlusions (Table I) attending the eye department, St Thomas's Hospital, were investigated. The group consisted of 25 men and 19 women with a mean age of 61.6 years (range 40-76). Twenty-three of the patients were investigated within the first week of presentation and 15 within a further four weeks. In six cases, however,

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Table I Summary of the 44 patients with retinal vein occlusion investigated

Patients	Central retinal vein (n=20)	Branch vein (n=24)
Women	9	10
Men	11	14
Mean age (years)	62.5	60.7

the patient was unaware of symptoms and the diagnosis was made on ophthalmoscopy.

The group was compared with a group of 30 controls consisting of 18 men and 12 women with a mean age of 57.9 years (range 45-75) taken from hospital personnel and ophthalmic patients who had neither inflammatory nor vascular conditions of the eye. Possible controls with any history or evidence of hypertensive, cardiovascular, or respiratory disease were rejected. A younger control group of 30 hospital personnel (mean age 30.7 years; range 20-44) was also examined but the results were not used for statistical comparison with the RVO group for reasons discussed later.

COLLECTION AND PREPARATION OF BLOOD

Blood samples were taken without venous occlusion during sampling and at the same time of day (1400 ± 1 hour) from both the controls and the patients, who were ambulatory immediately before venesection. The samples were subdivided for estimation of whole blood and plasma viscosity, fibrinogen, haematological examination, and biochemical analysis.

VISCOSITY MEASUREMENTS

These were carried out within seven hours of collection on lithium heparinized blood (20 U/ml) which was mixed continuously at room temperature during the interval before measurement. Whole-blood viscosity was measured at four shear rates. A Wells Brookfield cone-plate micro-viscometer (Model LVT) was used to measure viscosity at a shear rate of 230 inverse seconds (s^{-1}). A Contraves Low Shear 2 co-axial cylindrical type viscometer was used for measurements at shear rates of 23, 2.62, and 0.77 s^{-1} . Plasma viscosity

was measured with a Harkness Mk 2 capillary viscometer. All measurements were made at 37°C against a standard control (Brookfield viscosity standard). Estimations were all duplicated and the mean value taken.

Yield stress values were obtained by constructing a Casson plot (Casson, 1959) of the square root of the shear stress against the square root of the shear rate. Using shear stress values calculated at shear rates of 2.62 s^{-1} and 0.77 s^{-1} a line was extrapolated which gave the yield stress where the Y axis was intercepted. Merrill and others (1965) suggested that a straight linear relationship did not hold for values of shear rate less than 1 s^{-1} , but thought that this was probably owing to difficulty in interpreting viscosity results at low shear rates. We found, in separate experiments, that with the Contraves LS2 viscometer, which has a rapid response time, the relationship was linear to values of shear rate less than 1 s^{-1} (down to 0.488 s^{-1}).

HAEMATOLOGICAL INVESTIGATIONS

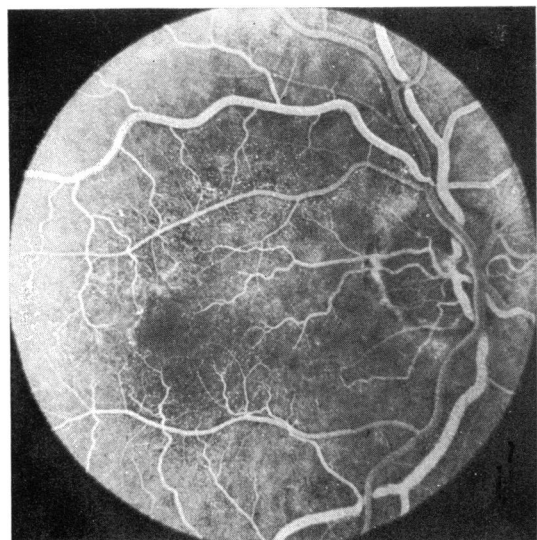
Routine haematological values were measured on a Coulter Counter Type S apparatus and platelet counts on a Coulter Counter Type Fn. Particular attention was paid to the packed cell volume (PCV), which was measured separately by us by a microhaematocrit technique in the heparin samples used for whole-blood viscosity measurement. Trapped plasma was measured on a further heparin sample by a ^{125}I -labelled albumin dilution technique (Garby and Vuille, 1961). Observed PCVs were adjusted to the correct PCV by subtracting the measured trapped plasma value. A blood film in each case was examined by one of us (TCP). ESR was measured at one hour by the Westergren method. Plasma fibrinogen was measured by the method described by Ingram (1952).

CHEMICAL DETERMINATIONS

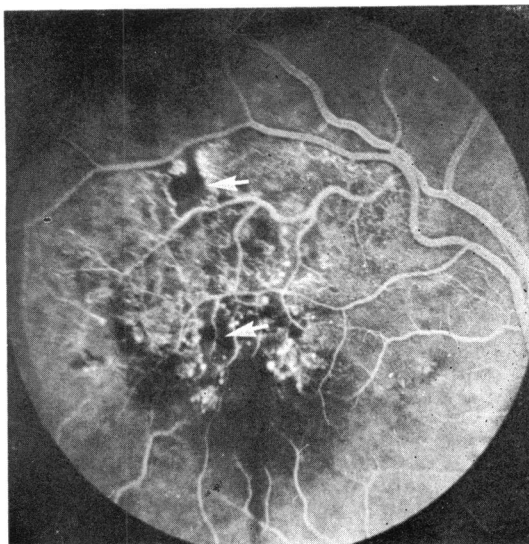
Serum protein, uric acid, and creatinine levels were determined by a routine multichannel analyser (Technicon SMA 12/60). Immunoglobulins (IgG, IgA, IgM) were estimated using immunodiffusion plates. RVO patients also had routine investigation of serum cholesterol, antinuclear factor, and serum complement. Fasting plasma triglycerides were estimated and a glucose tolerance test was carried out within the next week.

Table II Classification of 44 RVO patients into three groups (A, B, C) on the basis of the fluorescein angiographic appearances of the retina. The modified classification of branch vein occlusion as suggested by Archer and others (1974) is shown for comparison

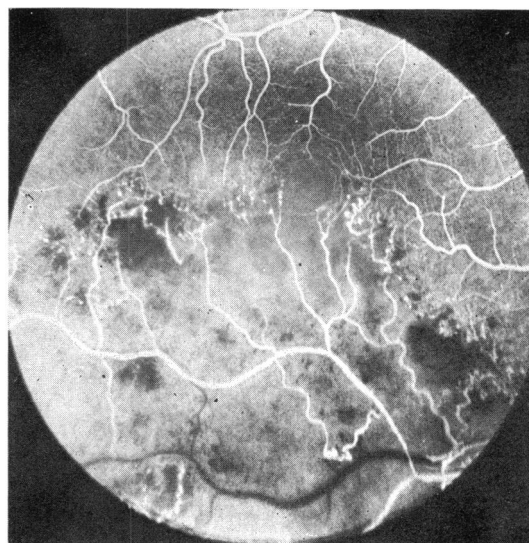
RVO group classification	BVO classification (Archer and others)	Definition	CRVO	BVO	Total
A	1 2	No significant areas of retinal ischaemia	12	5	17
B					
	3, subgroup A	Focal areas of capillary non-perfusion	2	5	7
C	3, subgroup B 4	Extensive areas of capillary non-perfusion	6	14	20



(1a)



(1b)



(1c)

FIG. 1 *Branch vein occlusions. (a) Group A. Dilated capillaries in distribution of a small superior temporal occlusion. (b) Group B. Small areas of capillary non-perfusion (arrows) in a superior temporal occlusion. (c) Group C. Widespread capillary non-perfusion in an inferior temporal occlusion. Note irregularity of calibre and leakage from arterioles passing through area*

BLOOD PRESSURE

When each subject was first seen the blood pressure was taken with the patient seated and by the same investigator (CPR). Diastolic pressure was taken as the point of muffling of Korotkoff's sounds. Blood pressure differential between the two arms was excluded in the RVO group. Patients with a diastolic pressure above 95 mmHg were considered to be hypertensive.

OPHTHALMIC EXAMINATION

Every patient in the RVO group was assessed on each visit with measurements of corrected visual acuity, slit-lamp examination, and applanation tonometry. Colour photographs (Kodachrome II) were taken and fluorescein angiography performed at presentation and on at least one other occasion. For fluorescein angiography 5 ml of 20 per cent sodium fluorescein was injected into an antecubital vein and the transit of dye through the fundus was recorded at 1-second intervals with a Zeiss fundus camera (Ilford FP4 film).

For ethical reasons fluorescein angiography was not performed on the control group.

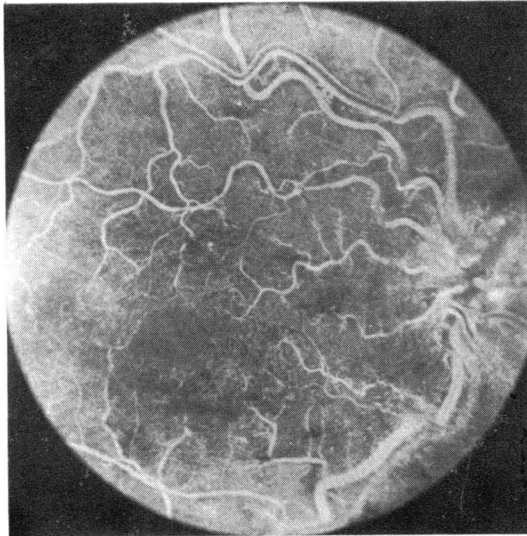
STATISTICS

Student's *t* test was used for comparisons of the groups.

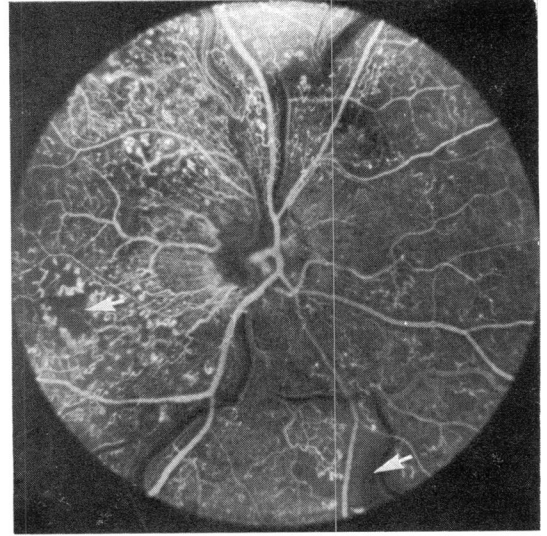
Results

GROUPING RVO PATIENTS

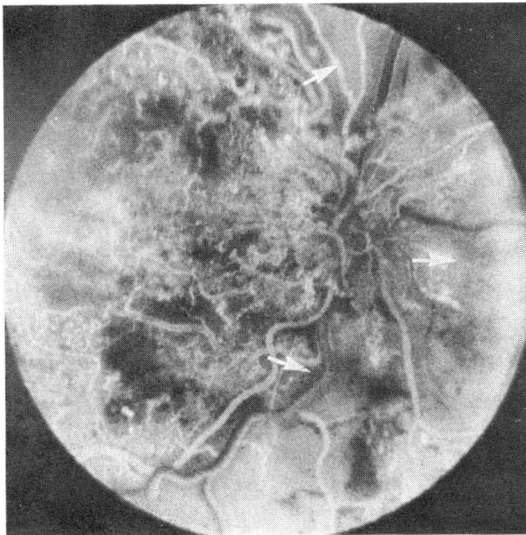
Archer, Ernest, and Newell (1974) classified their branch retinal vein occlusion (BVO) patients into four major groups (1-4), subdividing group 3 into 3A and 3B according to the results of a number of investigations by fluorescein angiography and suction cup ophthalmodynamometry. We have included both central and branch vein occlusions, which are considered to have similar underlying pathology. We have used a simplified classification to subdivide the whole RVO group purely on the



(2a)



(2b)



(2c)

FIG. 2 *Central vein occlusions. (a) Group A. An intact capillary bed with widespread dilatation. (b) Group B. Small areas of capillary non-perfusion (arrows). (c) Group C. Extensive capillary non-perfusion (arrows)*

fluorescein angiographic appearances, which were assessed for the presence and extent of capillary non-perfusion. Table II shows how the 44 RVO patients were subdivided into three groups, with the definitions and classification of Archer and others (1974) shown for comparison. Figs 1 and 2 are fluorescein angiograms illustrating an example of each subdivision in branch vein occlusion and central vein occlusion respectively.

Although all care was taken, an obvious source of inaccuracy with this method is observer error in assessing the capillary non-perfusion. All patients were subdivided before the significance

of the viscosity results was known. Subsequent statistical comparison of the RVO group was made in order to eliminate observer error—that is, group A was compared against group C, then groups A and B against group C, then groups B and C against group A.

VISCOSITY MEASUREMENTS

Whole-blood viscosity measured on the collected blood samples (that is, at the observed PCV) showed no statistical difference between the total retinal vein occlusion group and the control groups, at all shear rates. However, there was a highly significant difference ($P < 0.01$ at 230 s^{-1} and $P < 0.001$ at 23, 2.62, and 0.77 s^{-1}) between the control group and those patients with large areas of capillary non-perfusion (group C). Analysis within the RVO group itself showed a highly significant statistical difference between group A and group C ($P < 0.001$ at all four shear rates). Combination of group B with either A or C made little difference ($P < 0.001$ in six of the analyses and $0.001 < P < 0.005$ in the other two). Fig. 3 illustrates the viscosity results in the RVO and control groups at shear rates of 230 s^{-1} and 0.77 s^{-1} .

These findings indicate that the whole-blood

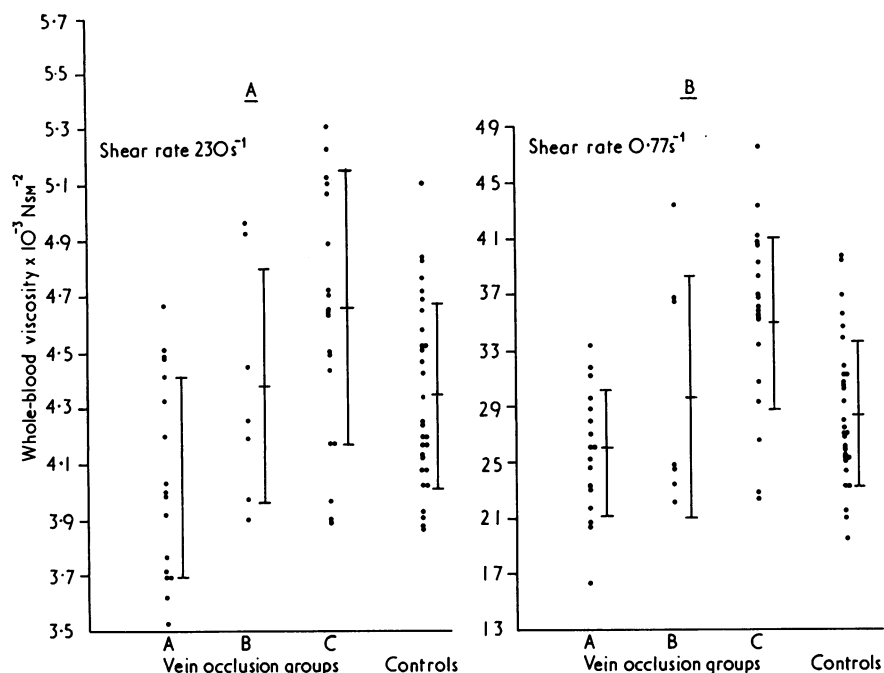


FIG. 3 Whole-blood viscosity results of RVO groups and controls at PCV at presentation at (a) shear rate of 230 s^{-1} , and (b) shear rate of 0.77 s^{-1} . Mean and one standard deviation are indicated ($P < 0.001$ for difference between groups A and C)

viscosity measured at the observed PCV was abnormal only in the RVO groups with capillary non-perfusion. If the RVO group is taken as a whole the abnormality was concealed by the normal viscosity of RVO patients without capillary non-perfusion.

Dormandy (1970) introduced the idea of using a regression line relating log viscosity values and PCV to 'correct' the viscosity results at the observed PCV to viscosity values at a mean PCV level. This eliminates the dominant PCV influence on whole-blood viscosity and allows analysis of the other major influencing factors, plasma protein composition and red cell deformability. Mean PCV values for the control and retinal vein occlusion groups were 0.431 and 0.426 respectively. Viscosity results were 'regressed' to a PCV of 0.42 , which allows for deducting 0.005 for trapped plasma. Comparison of whole-blood viscosity in the RVO group against the control group at this PCV level showed that it was higher in the occlusion group. Although this difference was not statistically significant at a shear rate of 230 s^{-1} ($0.05 < P < 0.1$) it became increasingly significant at lower shear rates ($0.005 < P < 0.01$ at 23 s^{-1} ; $P = 0.001$ at 2.62 s^{-1} ; and $0.001 < P < 0.005$ at 0.77 s^{-1}). Investigations within the RVO group on this basis showed a statistically significant difference ($0.01 < P < 0.02$) between the closure/non-closure groups at shear rates of 0.77 s^{-1} and 230 s^{-1} ($P = 0.025$). The results at 23 s^{-1} and 2.62 s^{-1} were higher but not

statistically significant ($0.05 < P < 0.1$). Fig. 4 shows the regressed viscosity results of the RVO and control groups at shear rates of 230 s^{-1} and 0.77 s^{-1} .

These results show that after the PCV effect on whole-blood viscosity had been eliminated other factors still influenced the results. The viscosity results for the total RVO group were higher than the control group. The patients with capillary non-perfusion had higher viscosity values than those without capillary non-perfusion.

Yield stress values were calculated from whole-blood viscosity measurements made at the observed PCV and there was no significant difference between the total RVO and control groups. However, analysis of group C against the controls showed a significantly higher yield stress value in group C ($P < 0.001$). When the RVO patients were considered significantly higher yield stress values were found between groups A and C ($P < 0.001$) and the statistical significance remained unaltered whether group B results were included with those of group A or C. Fig. 5 shows the yield stress values of these three groups and the controls for comparison.

These findings indicate that the yield stress calculated on the viscosity results at the observed PCV was abnormal in RVO patients with capillary non-perfusion. Taken within the RVO group as a whole this abnormality was concealed by the normal values of those without capillary non-perfusion.

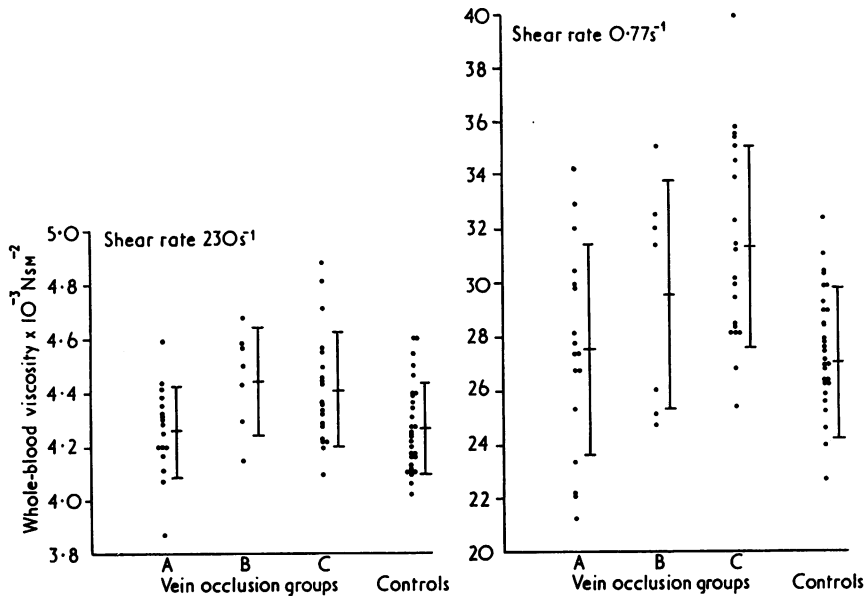


FIG. 4 *Whole-blood viscosity results in RVO and normal groups, after regression of viscosity results to a mean PCV of 0.42, at (a) shear rate of 230 s⁻¹, and (b) shear rate of 0.77 s⁻¹. Mean and one standard deviation are indicated (0.01 < P < 0.02 at 0.77 s⁻¹; P = 0.025 at 230 s⁻¹ for difference between groups A and C)*

After regression of the whole-blood viscosity values to the mean PCV (0.42) the yield stress values were recalculated, and there was no difference between the results of the control and total RVO groups. However, there was a highly significant difference ($P < 0.001$) when group C was

compared with the controls and also when the three RVO groups were analysed. Patients in group C had higher yield stress values than those in group A. Fig. 6 shows the yield stress values in the RVO groups and controls calculated from the whole-blood viscosity values at the mean PCV.

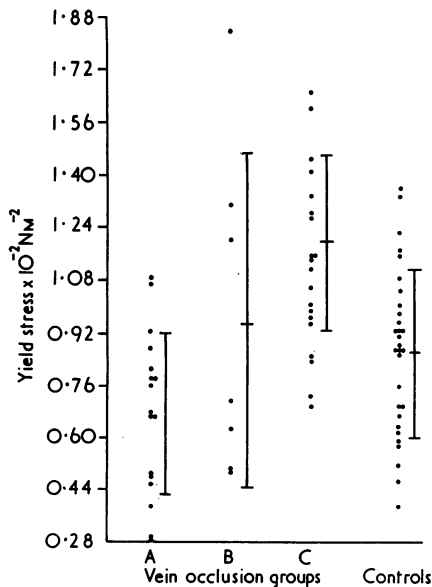


FIG. 5 *Yield stress values calculated at PCV at presentation for RVO and control groups. The mean and one standard deviation are indicated ($P < 0.001$ for difference between groups A and C)*

This shows that after eliminating the influence of PCV on whole-blood viscosity and recalculating the yield stress RVO patients with capillary non-perfusion still had abnormal results. Taken as a whole, however, the RVO patients did not have yield stress values significantly higher than the control group.

Plasma viscosity results showed a highly significant difference ($0.001 < P < 0.005$) between the values of the two differently aged control groups. Comparing the RVO and older control group there was also significant difference ($0.025 < P < 0.05$), but between the RVO groups there was no difference. This analysis correlates well with the fibrinogen results below.

These results indicate that although the RVO patients had an abnormal plasma viscosity it was no higher in the patients with capillary non-perfusion than in those without.

HAEMATOLOGICAL RESULTS

Although there was no significant difference between the trapped plasma results in the RVO and control groups, analysis of the PCV result was done on the measured PCV after subtraction of the trapped plasma. Over all there was no significant difference between the control and

total RVO groups ($0.4 < P < 0.5$). Further analysis allowing for variations of PCV between sexes did not alter the results. A highly significant difference, however, was found between groups A and C

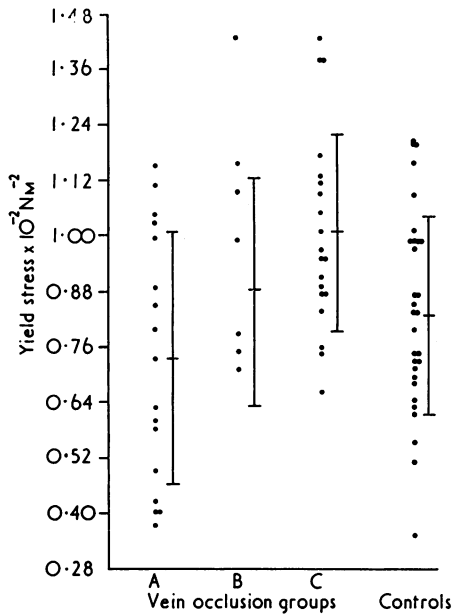


FIG. 6 Yield stress values calculated after regression of viscosity results to a mean PCV of 0.42. Mean and one standard deviation are indicated ($P < 0.001$ for difference between groups A and C)

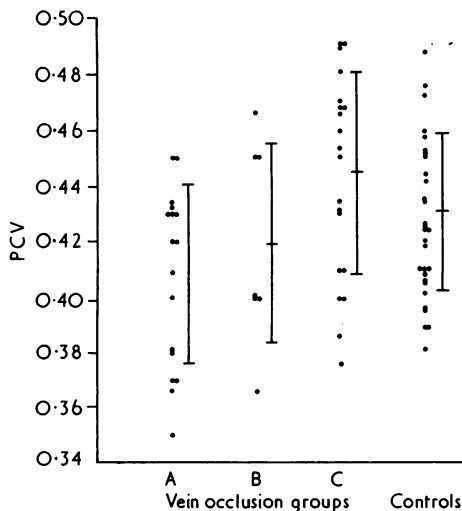


FIG. 7 PCV values within RVO and control groups. Mean and one standard deviation are indicated ($0.001 < P < 0.005$ for difference between groups A and C)

($0.001 < P < 0.005$). Including group B with either A or C produced the same significance. Fig. 7 shows the PCV results of the RVO and control groups.

These findings show that patients with capillary non-perfusion had a higher PCV than those without non-perfusion.

The plasma fibrinogen levels showed a highly significant difference between the younger and older control groups ($0.001 < P < 0.005$). Because of the important influence of fibrinogen on viscosity the younger group was not included in the statistical analysis. Comparison of the older controls

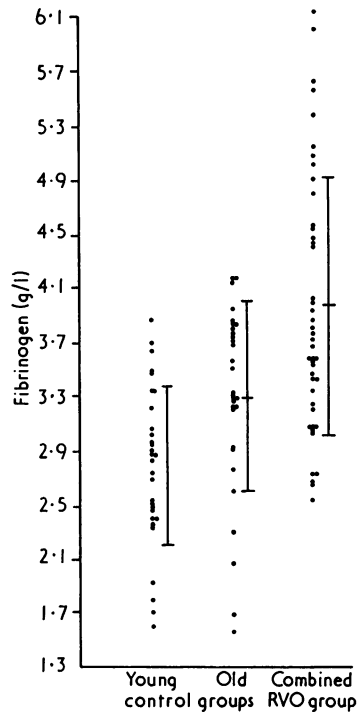


FIG. 8 Plasma fibrinogen values in control RVO groups. Mean age of young and old control groups was 30.7 and 57.9 years respectively. Mean and one standard deviation are indicated

with the RVO group also showed a significant difference ($P < 0.001$). There was no significant difference between the three RVO groups. Fig. 8 shows the relationship of the fibrinogen values.

These results indicate that plasma fibrinogen levels were abnormal in the RVO patients but the abnormality did not correlate with the presence or absence of capillary non-perfusion.

Other routine haematological investigations were normal in all control and patient groups, and in particular there were no patients or control subjects with a raised erythrocyte sedimentation rate. Blood films showed no evidence of any haematological disorder.

Table III Summary of further investigations in the RVO and control groups

Investigation	SI units		Traditional units		Significance
	RVO group		Controls		
	RVO group	Controls	RVO group	Controls	
Plasma viscosity	1.297 (0.07) $\times 10^{-3}$ Nsm ⁻²	1.263 (0.04) $\times 10^{-3}$ Nsm ⁻²	1.297 (0.07) cps	1.263 (0.04) cps	P < 0.05
Fibrinogen	3.98 (0.95) g/l	3.28 (0.60) g/l	397.8 (94.7) mg/100 ml	328.7 (69.4) mg/100 ml	P < 0.005
Uric acid	0.356 (0.087) mmol/l	0.321 (0.082) mmol/l	5.93 (1.36) mg/100 ml	5.35 (1.36) mg/100 ml	0.05 < P < 0.1
Creatinine	106.8 (27.4) mmol/l	91.9 (12.4) mmol/l	1.14 (0.31) mg/100 ml	1.04 (0.14) mg/100 ml	0.05 < P < 0.1
Total protein	72.6 (4.0) g/l	72.3 (3.0) g/l	7.26 (0.4) mg/100 ml	7.23 (0.3) mg/100 ml	NS
Immunoglobulins					
IgG	10.20 (1.00) g/l	10.25 (1.70) g/l	1020.6 (109.4) mg/100 ml	1025.3 (178.5) mg/100 ml	NS
IgA	2.35 (1.01) g/l	1.90 (0.64) g/l	234.6 (101.4) mg/100 ml	190.3 (63.8) mg/100 ml	P < 0.05
IgM	1.26 (0.62) g/l	1.21 (0.52) g/l	126.0 (62.0) mg/100 ml	121.4 (52.0) mg/100 ml	NS
Blood pressure					
Systolic	161.3 (30.1) mmHg	137.7 (12.6) mmHg	—	—	P < 0.001
Diastolic	94.1 (13.3) mmHg	87.3 (7.6) mmHg	—	—	P < 0.02
Trapped plasma	1.22 (0.07) per cent	1.21 (0.11) per cent	—	—	NS

NS = not significant

Table IV Additional investigations in the RVO group

Investigation	No. investigated	SI units		Traditional units		Significance A-C	Significance Laboratory range
		Group mean		Group mean			
		Group A (+1 SD)	Group C (+1 SD)	Group A (+1 SD)	Group C (+1 SD)		
Cholesterol	44	6.13 mmol/l	6.24 (1.15)	236.4 mg/100 ml	226.8 (51.6)	NS	100-240 mg/100 ml
Fasting triglyceride	40	1.346 mmol/l	0.97 (0.35)	119.1 mg/100 ml	85.7 (31.3)	P < 0.001	60-160 mg/100 ml
Complement C ₃	41	0.79 g/l	1.66 (0.64)	79.1 mg/100 ml	34.6 mg/100 ml		80-180 mg/100 ml
C ₄	10	0.35 g/l		34.6 mg/100 ml			20-50 mg/100 ml
Antinuclear factor	37	Negative					
	4	Positive 1:10					
	2	Positive 1:50					

NS = not significant

BIOCHEMICAL RESULTS

These are summarized in Table III. Serum uric acid levels were higher in the RVO group than the controls, and this became statistically significant when the control group was compared with group C ($0.01 < P < 0.02$). Serum creatinine, a more accurate indicator of renal function than urea values, was higher in the RVO group than in the controls although the difference was not statistically significant ($0.05 < P < 0.1$). Analysis of total serum proteins and serum globulins showed no significant differences between groups.

Immunoglobulin electrophoresis did not detect any dysproteinaemic state in the patients examined. IgG and IgM levels were not significantly different between the control and RVO groups but interestingly there was a significantly higher level of IgA in the RVO group ($0.025 < P < 0.05$).

Further investigations performed within the RVO group are summarized in Table IV, and show a high mean cholesterol level of 6.13 mmol/l (236.4 mg/100 ml) (range 3.76–8.94 mmol/l) (145–345 mg/100 ml). There were 24 patients with levels equal to or greater than our laboratory upper range of normal (6.22 mmol/l) (240 mg/100 ml).

Fasting triglycerides were estimated in only 40 RVO patients and 11 had levels equal to or greater than the upper limit of normal (1.81 mmol/l) (160 mg/100 ml). The mean level was 1.34 mmol/l (119 mg/100 ml) (range 0.45–2.66 mmol/l) (40–235 mg/100 ml).

C₃ complement levels were equal to or less than the lower limit of normal (0.8 g/l; 80 mg/100 ml) in 22 patients (range 0.47–1.18 g/l; 47–118 mg/100 ml). C₄ levels were measured in 10 of the patients with the lowest C₃ levels but these were all normal. Antinuclear factor titres were all normal ($< 1/10$) except for equivocally raised levels in six patients (Table IV).

OTHER DISORDERS IN RVO GROUP

Blood pressure differences between the control and RVO groups showed a highly significant difference at systolic levels ($P < 0.001$) and a significant difference at diastolic levels ($0.01 < P < 0.02$). Eight patients were receiving treatment for hypertension at the time of diagnosis. Hypertensive treatment was started in nine patients after presentation. Four patients had received hypotensive therapy in the past but three of them were normotensive at presentation. Twenty-six patients (59% of the total group) were therefore considered to be normotensive. The total number of patients receiving or in need of hypotensive treatment was 18, and 14 of these had evidence of some capillary closure.

Glucose tolerance tests detected one diabetic in the group. Various forms of cardiovascular disease were present—intermittent claudication (one patient), history of deep vein thrombosis (three patients), myocardial infarct (one patient), and angina (one patient). One patient had had a bowel carcinoma resected three years previously.

Discussion

Before discussing our results we must comment on certain aspects of methodology.

PCV and hence whole-blood viscosity are influenced by venous stasis during collection of blood samples (Berry, Perkins, and Jernstrom, 1950) and by posture and mobility (Widdowson and McCance, 1950), and they are also subject to diurnal variation (Ehrly and Jung, 1973). Venous occlusion was therefore avoided and, so far as possible, all subjects were comparable in terms of posture and mobility when blood was taken. Ehrly and Jung (1973) emphasized the importance of sampling between 1 pm and 5 pm, when diurnal variations in factors affecting viscosity are most constant. We therefore used this period in our study.

Anticoagulation of the blood for viscosity measurement complied with the observation of Zingg, Sulev, and Morgan (1973), who found no difference in whole-blood viscosity in samples with heparin concentrations varying from 14–95 U/ml. Blood viscosity is critically temperature-dependent (Rand, Lacombe, Hunt, and Austin, 1964) and for this reason all measurements were done at a body temperature of 37°C.

The influence of age on PCV (Mayer, 1964) and fibrinogen (Ditzel and Kampmann, 1971) made it necessary to divide the 60 normal subjects into two groups, and 30 over the age of 45 years were used as a control group. Although the patient group and control group were not precisely matched for age, the mean age of 61.6 years of the RVO group compared closely with that of 57.9 years of the control group. The mean age in the RVO group collates well with other studies (Blankenship and Okun, 1973, 62.6 years; Michels and Gass, 1974, 63 years; Clemett, Kohner, and Hamilton, 1973, 59.5 years; Gutman and Zegarra, 1974, 57.5 years). There was no statistical difference between the mean ages of patients with and without capillary non-perfusion. However, they differed in PCV, whole-blood viscosity, and yield stress values. The ratio of men to women in the patient and control groups was about the same—namely, M:F 25:19 and 18:12 respectively.

Capillary viscometers with their inconstant shear rate have been used for many years to show differences in whole-blood viscosity. Wells, Denton, and

Merrill (1961) introduced a modification of a cone-plate viscometer to measure whole-blood viscosity. With the ability to control the shear rate interest has been focused on changes in blood constituents that might lead to an alteration in the rheological (flow) characteristics of blood. The question of whether increased blood viscosity is an aetiological factor in the development of thrombosis at sites other than the retinal circulation has been discussed by many authors. There is no doubt that retinal vein occlusion is a relatively common feature in conditions characterized by a hyperviscosity state—for example, polycythaemia (Duke-Elder, 1967) and macroglobulinaemia (Spalter, 1959). These conditions should obviously be sought in every patient developing a retinal vein occlusion or, of course, an occlusion at any other site. A hyperviscosity state, however, was not present in any of our patients. Some had raised immunoglobulin levels, but none high enough to cause hyperviscosity, and no patient had a PCV exceeding 0.50.

In this study higher levels of fibrinogen, plasma viscosity, and whole-blood viscosity, particularly at low shear rates, were found in the total patient group, especially in those with capillary non-perfusion, compared with the control group. The possibility that these abnormalities contribute to occlusion requires consideration. Ott and others (1974) found higher blood-viscosity values, more obviously significant at low shear rates, in patients with cerebral infarction compared to an age-matched control group. Blood viscosity measured at low shear rates was many times higher in patients suffering from arterial or venous thrombosis than in healthy individuals (Dintenfass, 1966). In a prospective study of postoperative venous thrombosis Dormandy and Edelman (1973) found a significant relationship between the development of thrombosis and raised blood viscosity and fibrinogen levels. They measured viscosity only at a shear rate of 230 s^{-1} , and probably a more striking difference would have been seen if they had measured viscosities at low shear rates. In veins the shear rate is lower than in other parts of the circulation (Whitmore, 1967; Dintenfass, 1971). Fibrinogen and the globulin fraction of blood are responsible for the formation of red cell aggregation (Chien, Usami, Dellenbach, and Gregerson, 1970), and it is this phenomenon that imparts the non-Newtonian behaviour of blood (Chien, Usami, Dellenbach, and Gregerson, 1967) with a rise in whole-blood viscosity at low shear rates.

The presence of a definite venous thrombosis in retinal vein occlusion has been questioned (Kohner and Shilling, 1976) and, indeed, the course of events still remains obscure. A consideration of the rheological events at the time of occlusion may add further insight into the pathophysiology of the

condition. Fujino and others (1968) showed experimentally that complete venous occlusion produced arterial stagnation and an ischaemic retina, and that only partial occlusions produced a similar picture to that of CRVO. Furthermore, there is always evidence of some venous flow as shown by fluorescein angiography (Fujino and others, 1968; Oosterhuis, 1969). Similar clinical evidence supports the view that complete venous occlusion is only rarely observed in branch vein occlusion (Kohner and Shilling, 1976), although the damage to the vein wall and endothelial proliferation is an accepted pathological finding. The fact that a venous thrombosis partially occluding the lumen may occur but not lead to complete obstruction remains a possibility. A controlled randomized study of the role of a fibrinolytic agent, streptokinase, in CRVO concluded that it was not beneficial (Kohner, Hamilton, Bulpitt, and Dollery, 1974). This supports the suggestion that a venous thrombosis does not occur or, alternatively, that the introduction of streptokinase was too late after the occlusion for the patients to benefit from it.

Pathological changes in the vasculature of the retinal circulation are almost certainly a factor in the development of RVO. In BVO impedance of blood flow is virtually always at arterio-venous crossings (Gass, 1968) where there is either pressure on the vein or a thickened wall due to arterial disease or endothelial proliferation, or both. Similar pathological processes probably occur at the lamina cribrosa in CRVO but absolute confirmation is more difficult to obtain. Fluorescein angiography provides the proof of turbulence at arterio-venous crossings in BVO and on pathological grounds it is reasonable to expect a similar situation in CRVO.

Even when blood viscosity is normal a 'triggering mechanism' at the site of 'occlusion' might set off a rheological vicious circle, the decrease in flow (shear rate) causing an increase in blood viscosity owing to the non-Newtonian behaviour of blood. This would be followed by a further decrease in flow with a vicious circle type of increase in viscosity. Increase in viscosity has also been shown to occur with a reduction in pH (Murphy, 1967), and this might also occur locally after the initial period of slowing, further increasing viscosity. Rheological occlusion seems a possibility and this may or may not be followed by a true thrombosis. A 'rheologic obstruction' at the isometric phase of ventricular contraction, when the shear rate falls almost to zero, was suggested by Burch and De Pasquale (1965) as the cause of myocardial infarction in the absence of an anatomical obstruction of the coronary arteries. Retinal vein occlusion may be another example of 'rheological obstruction'. The triggering mechanism to the process is difficult to explain in

terms of blood viscosity alone except perhaps in patients with much higher values. To study the possibility that higher blood-viscosity values predispose to the development of retinal vein 'occlusion' it would be necessary to do a prospective study on a group of patients with a similar degree of retinal vessel changes and without occlusion. Blood viscosity and the retinal appearances would need to be followed for a time. Finally, the group would be subdivided into those with and those without vein occlusion. Comparison of the viscosity values of the two groups would then give a clearer picture of the role of this factor.

The most striking finding in the present study was that of increased values for PCV and whole-blood viscosity in patients with capillary non-perfusion compared with those without. The precise mechanism of capillary non-perfusion remains obscure. Ashton (1970) mentions that in capillary non-perfusion the capillaries may not in fact be closed but that a state of circulatory stagnation may exist. This hypothesis seems very plausible in a situation where the viscosity of blood is increased. RVO patients have already been shown to have a higher viscosity than the control group, yet among these patients there is a group with even higher values. The fact that this is the group showing capillary non-perfusion suggests a close link with the theory of Ashton (1970). The vicious circle situation mentioned above might in the presence of higher viscosity predispose to a situation of circulatory stasis in some of the retinal capillary bed. The magnitude of the force required to re-establish flow (yield stress) in a stationary blood column would become critical, and it must be significant that this value is also higher in patients with non-perfusion than without. When capillary non-perfusion is present the distribution is usually patchy, and this probably represents local variation in shear stress and shear rate.

The importance of capillary non-perfusion has been investigated by Shilling and Kohner (1976). They found a statistically significant association between capillary non-perfusion and retinal neovascularization which is important in the morbidity of RVO due to vitreous haemorrhage, preretinal fibrosis, and retinal detachment. Hence patients with higher viscosity and yield stress values may well be at a greater risk of a more serious event with the development of capillary non-perfusion, retinal ischaemia, and its complications.

Our study shows therefore that differences in blood constituents may affect the outcome. This factor was considered in a study of acute myocardial infarction by Hershberg, Wells, and McGardy (1972), who found that there was no correlation between the PCV after admission to hospital and the eventual outcome of the episode.

Viscosity values were not, however, measured, and Dormandy (1970) has commented that the PCV might not be a reasonable guide to the whole-blood viscosity value in a particular individual. Furthermore, acute myocardial infarction may be characterized by considerable systemic disturbance that might influence the value obtained for the PCV on a sample of blood taken after the infarction. Retinal vein occlusion is associated with no systemic disturbance.

Variation of PCV at different sites in the circulation is well recognized. The possibility arises that the PCV, and hence viscosity of a sample of blood taken from an antecubital vein, may not accurately reflect the PCV prevailing in the 'occluded' retinal vein. Therefore the dominant PCV influence on the whole-blood viscosity was eliminated and other factors influencing viscosity were analysed. This was done by the method of Dormandy (1970) where a log viscosity/PCV correlation equation was used to compare all results 'regressed' to the same mean PCV of 0.42. Significantly higher values were found for yield stress and whole-blood viscosity after carrying out this regression procedure when patients with capillary closure were compared to those without capillary closure.

There is general agreement that arterial vascular disease is commonly present in retinal vein occlusion (Braendstrup, 1950; Paton, Rubinstein, and Smith, 1964; Kohner, 1964; Raitta, 1965; Clements, Elsby, and Smith, 1968; McGrath and others, 1973; Gutman and Zegarra, 1974; Michels and Gass, 1974). This is supported by our observations that 41 per cent of the patients were receiving or required treatment for hypertension and that there were histories of angina (one patient), intermittent claudication (one patient), and myocardial infarction (one patient). Although analysis of the recorded blood pressures showed a statistical difference between the control and RVO groups it must be taken into account that the control group was selected to exclude hypertension. Comparison of recorded blood pressures between the RVO groups showed no statistical difference. The association of hypertension and capillary non-perfusion was also investigated using the χ^2 test. No significance between these two pathological changes could be found ($P=0.118$) in our relatively small number of patients. A larger survey would probably decide whether there was any association.

Hyperlipidaemia is recognized as a factor in the development of arterial disease (Lewis, Chait, Wootton, Oakley, Krikler, Sigurdsson, February, Maurer, and Birkhead, 1974). Comparison between cholesterol levels in patients with and in those without large areas of capillary non-perfusion showed no difference, although the mean for the total group was only just within the upper limit for

our laboratory (Table III). A significant difference ($P < 0.001$) was found in the fasting triglyceride levels comparing groups A and C. Hyperuricaemia has been linked with the development of arterial disease (Kannel, Dawber, Friedman, Glennon, and McNamara, 1964). Our total patient group compared with the controls had a higher serum uric acid. This does not reach statistical significance at the 5 per cent level but does so if group C is compared with the control group. In a group of patients with a high incidence of hypertension a relatively reduced level of renal function might be expected. Our patient group had a higher serum creatinine level than the controls, although this did not reach a statistically significant level ($0.05 < P < 0.1$).

These biochemical investigations support the strong association of arterial disease and retinal vein occlusion. They also suggest that the incidence of biochemical abnormalities is higher in those patients with capillary non-perfusion, which supports the recent observations of Shilling and Kohner (1976).

The absence in every patient of immunoglobulin changes compatible with a hyperviscosity syndrome has been commented upon. It is interesting, however, that a significantly higher IgA level was found between the control and the total patient group. The mean level of IgA in the retinal vein group, however, was not higher than the upper expected limit for our laboratory (4.0 g/l). McGrath and others (1973) found raised IgA levels in 14 of their 54 patients with retinal vein occlusion aged over 50 years. The actual levels found in their series were not mentioned. The finding of a higher IgA level in the retinal vein group remains unexplained.

Ellis, Hamer, Hunt, Lever, Lever, Peart, and Walker (1964) reported an association of neoplasia and systemic lupus erythematosus with retinal vascular occlusion. Our series does not show such a high incidence of neoplasia (only one patient) and ANF titres were all negative ($< 1/10$) except for equivocally raised levels in six patients (see Table III). Diabetes (Cohen, Staunton, Irving, and Gorchein, 1973) and glaucoma (Dryden, 1965) have been associated with retinal vein occlusion. Our finding of one patient with diabetes and two patients with raised intraocular pressure confirms the importance of seeking these conditions.

This study of blood and plasma viscosity has given a possible insight into the pathophysiology of the condition of retinal vein occlusion. The question of whether a therapeutic measure to alter the blood constituents could modify and moderate the morbidity of the condition is very difficult to assess. Possibly irreversible retinal

damage, if it is going to occur, has already occurred before the patient presents. From a rheological stand point the only therapeutic measures available are venesection to lower the PCV, the use of an agent to lower the fibrinogen level, and treatment of hypertension if present. Clofibrate has been used by Dormandy, Gutteridge, Hoare, and Dormandy (1974) in patients with intermittent claudication and has been shown to lower both fibrinogen and whole-blood viscosity. Therapeutic phlebotomy has been shown by Burch and De Pasquale (1963) to benefit patients with angina pectoris, but they treated patients who had a PCV exceeding 0.50. This feature was not present in any of our patients.

Retinal vein occlusion may occur more than once in an individual. It would therefore seem reasonable that there should be a controlled study to determine whether such treatment reduces the incidence of further occlusion in patients with RVO who have a rheological abnormality which can be modified by treatment.

Summary

Whole-blood and plasma viscosity with haematological and biochemical investigations were measured in 44 patients with retinal vein occlusion. The patients were subdivided on the basis of fluorescein angiographic findings into:

1. Those with large areas of capillary non-perfusion.
2. Those with small areas of capillary non-perfusion.
3. Those with an intact capillary pattern.

Capillary non-perfusion in retinal vein occlusion is associated with a higher morbidity owing to the complications of retinal neovascularization. Significantly higher values of whole-blood viscosity, packed cell volume, and yield stress have been found in patients with capillary non-perfusion than in those without. These differences may be of critical importance during the episode of retinal vein occlusion and suggest an aetiological factor in the development of capillary non-perfusion. Higher whole-blood and plasma viscosity values and plasma fibrinogen levels have also been shown in the whole retinal vein occlusion group compared with a control group of 30 individuals. These differences may be a factor in the development of retinal vein occlusion but their precise role is difficult to evaluate.

Further biochemical investigations in the vein occlusion group supported the strong association with arterial disease and suggested a higher incidence of biochemical abnormalities in those patients with capillary non-perfusion.

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