PLASMA VISCOSITY*

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The relationship between the blood sedimentation rate and plasma viscosity was first observed by Fåhreus in 1921, when investigating the mechanism of the blood sedimentation test. He did not, however, pursue the subject further, and it was not till 1940 that plasma viscosity was first suggested as a clinical test by T'ang and Wang. These workers studied a number of samples from patients suffering from pulmonary tuberculosis and concluded that plasma viscosity gave more useful information than the E.S.R. as to the activity of the disease process. About the same time Miller and Whittington (1942) were also studying the value of plasma viscosity in pulmonary tuberculosis, and their conclusions were similar. The plasma viscosity is essentially a measure of the plasma protein changes, and particularly of those proteins which are the most hydrated in colloid suspension, namely fibrinogen and euglobin (Chick, 1914). The relative importance of the individual protein changes in disease may be elucidated by means of a fractional viscosity technique.

Method

Blood is collected in an ammonium potassium oxalate† tube, and the plasma separated by centrifuging. The viscosity of the plasma is then determined. For this purpose an Ostwald viscometer was used, but any instrument of appropriate capacity is suitable. The Ostwald instrument consists essentially of two bulbs connected by a U-shaped capillary tube (Fig. 1). The plasma is sucked into the upper bulb and is then timed as it flows through the capillary tube into the lower bulb, that is from mark C to mark G. This is compared with the time taken for water. The temperature used in these tests was 25° C. The viscosity is calculated according to the formula $v=\frac{T_1}{T_2}\times 100$, where T_1 is the time taken for plasma and T_2 the time taken for water.

Having estimated the viscosity of the plasma, this is then clotted by the addition of 0·025 ml. of 40 per cent. calcium chloride for each 0·1 ml. of oxalate mixture. It is then placed in the incubator for half an hour. The clot is removed and the serum extruded from it ;

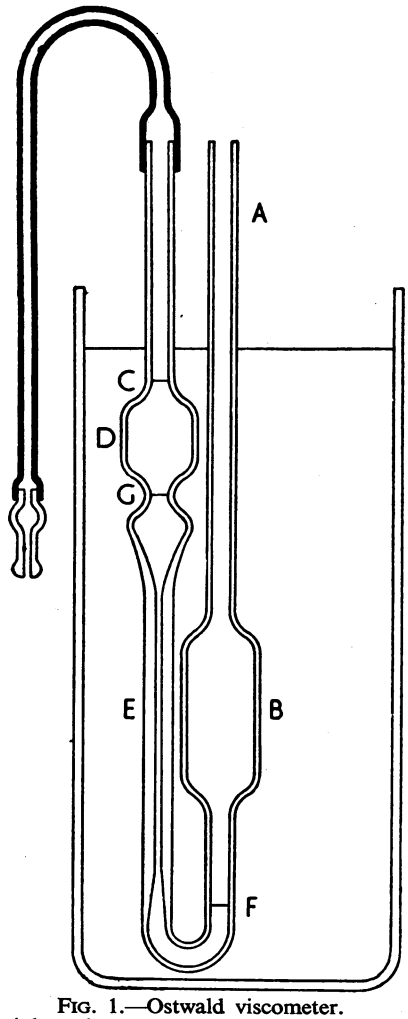

FIG. 1.—Ostwald viscometer.
A = inlet tube.
B = lower reservoir.
C = upper level of fluid at commencement of test.
D = upper reservoir.
E = capillary tube.
F = lower level of fluid at commencement of test.
G = upper level of fluid on completion of test.
The subsidiary bulb below mark G may be smaller and can be omitted altogether without serious detriment, thus reducing the amount of plasma required.

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† Ammonium oxalate 6 g., potassium oxalate 4 g., water to 100 ml. This solution must be kept in an incubator at 37° C. to avoid crystallization. 0·1 ml. is required for 10 ml. of blood.

the viscosity of this serum is determined. To one volume of the serum, a half volume of 4 M ammonium sulphate is then added, and the resulting globulin precipitate is filtered off. This precipitate is almost entirely gamma globulin (Jager and Nickerson, 1948). The viscosity of the filtrate is then determined and the remainder of the globulin is precipitated by the addition of a third-volume of 4 M ammonium sulphate to each volume of filtrate. The precipitate is filtered off and the viscosity of this final filtrate is estimated.

In this way four figures are obtained. An example is quoted :

	Plasma	Serum	1st filtrate	2nd filtrate	(water = 100)
Original viscosity figures	157	143	139	144	
Viscosity differences due to ammonium sulphate	—	—	16	27	
Corrected viscosity figures	157	143	123	117	
	Fibrinogen	Globulin I	Globulin II	Albumin	
Fractional viscosity differences	14	20	6	17	

As the filtrate contains ammonium sulphate which has an appreciable viscosity in solutions of these concentrations, a deduction must be made to allow for the viscosity of the salt solution. As the viscosity of an ammonium sulphate solution in the concentrations present in the filtrates is 116 and 127 respectively, the viscosity difference of 16 is deducted from the figure of the first filtrate and 27 from the viscosity of the second filtrate. By deducting these from the original figures a series of corrected viscosity figures are obtained. Thereafter by deducting the serum from the plasma viscosity the fibrinogen viscosity difference is calculated. In the same way by deducting the corrected viscosity of the first filtrate from the serum viscosity figure the first globulin viscosity difference is obtained, and in like manner the second globulin difference from the first and second filtrates. To convert the figure for albumin into a viscosity difference, 100, that is the value for water, must be deducted from the viscosity of the second filtrate. The method of calculation, though empirical, does in fact give figures closely similar to the published results of the viscosity of pure protein solutions, and is found to give a value approaching zero when negligible amounts of precipitate are present. There is, however, a slight error due to the effect of the salt on the viscosity of protein solutions. For example, by the addition of an increasing amount of ammonium sulphate to plasma it may be shown that up to a concentration of 1 per cent. of the salt there is a reduction of viscosity, allowing for the viscosity of the contained salt. This amounts to 1-4 in normal serum and up to 9 in pathological samples. Beyond this any further addition of ammonium sulphate does not result in a reduction of viscosity until precipitation of globulin starts at 25 per cent. saturation. The viscosity difference due to the first globulin fraction is thus made up partly of this initial salting factor, and a more accurate figure may be obtained by deducting this. In practice, however, it has been found that the introduction of this extra step into the method yields no additional information and it has therefore been abandoned.

Normal Values

Figures obtained in forty-five healthy males and females were as follows : fibrinogen 5 to 18 ; globulin I 12 to 25 ; globulin II 2 to 10 ; albumin 17 to 25.

It should be observed that these are the values obtained with the instrument described above and would not necessarily be correct for viscometers not of identical design. No significant age or sex difference was observed.

Incidence of Viscosity Changes

The incidence of the changes found in disease is shown in Table 1. The most frequent finding

TABLE 1

INCIDENCE OF FRACTIONAL VISCOSITY PATTERNS IN 213 ABNORMAL SAMPLES

	Value	%
Fibrinogen only increased	38	18
Globulin I only increased	37	17
Fibrinogen and globulin I increased	30	14
Fibrinogen and globulin II increased	12	6
Fibrinogen and globulin I and II increased	12	6
Albumin alone reduced	13	6
Globulin II only increased	10	5
Fibrinogen increased and albumin reduced	8	4
Fibrinogen and globulin I and II increased and albumin reduced	9	4
Fibrinogen and globulin II increased and albumin reduced	6	3
Globulin II increased and albumin reduced	4	2
Fibrinogen and globulin I increased and albumin reduced	4	2
Globulin II reduced	5	2
Globulin I and II increased	2	1
Globulin I reduced	2	1
Globulin I and albumin reduced	3	1
Fibrinogen increased and globulin I reduced	2	1
Globulin II increased, globulin I reduced, and albumin reduced	2	1
Globulin I and II, and albumin reduced	1	0·5
Globulin I and II increased and albumin reduced	1	0·5
Fibrinogen and globulin II increased, globulin I and albumin reduced	1	0·5
Globulin II increased, globulin I and albumin reduced	1	0·5
Globulin I increased and albumin reduced	—	0

TABLE 2

ACUTE DISEASE DURING THE FIRST WEEK

Normal	Day of disease	E.S.R. 2-15	Fib. 5-18	Glob. I 12-25	Glob. II 2-10	Alb. 17-27	Total 44-71
Bronchitis	5	9	22	26	12	18	78
Pneumonia	3	80	46	19	10	17	92
,, ...	3	65	30	13	—	—	66
,, ...	3	30	30	18	—	—	81
,, ...	4	98	45	18	—	—	94
Rheumatic fever	3	80	46	21	—	—	102
,, ,, ...	6	4	30	26	—	—	84
Salpingitis	3	13	16	14	14	22	66
Tuberculous pleurisy ..	4	32	34	23	—	—	88
,, ,, ...	3	78	40	12	—	—	86
Influenza	5	36	29	23	—	—	77
Coronary thrombosis ..	6	10	39	18	—	—	87
Erythema multiforme ..	2	86	42	19	16	17	94

TABLE 3

ACUTE DISEASE, SECOND WEEK

Normal	Day of disease	E.S.R. 2-15	Fib. 5-18	Glob. I 12-25	Glob. II 2-10	Alb. 17-27	Total 44-71
Appendicitis followed by pylephlebitis	8	49	64	34	13	21	132
Pneumonia	8	6	24	24	9	17	24
Rheumatic fever	—	50	39	27	9	24	99
,, ,, ...	12	12	28	30	—	—	87
,, ,, ...	14	46	44	32	—	—	106
Influenza	10	57	26	24	7	14	71

TABLE 4

CONVALESCENT PATIENTS

Normal	Day of disease	E.S.R. 2-15	Fib. 5-18	Glob. I 12-25	Glob. II 2-10	Alb. 17-27	Total 44-71
Lupus erythematosis (sub-acute)	120	23	17	37	6	22	82
,, ,, ,,	128	20	14	30	—	—	71
Pneumonia (acute)	21	11	16	28	9	21	74
,, ,, ...	40	9	14	26	4	21	65
Pneumonia (sub-acute) ..	90	7	18	26	4	20	68
,, ,, ..	30	13	13	29	5	22	69
Rheumatic fever	—	—	13	27	4	21	65
,, ,, with carditis	75	12	17	33	—	—	78
Tuberculous pleurisy ..	110	5	18	27	6	20	71
,, ,, ..	130	13	16	29	—	—	67

was an increase of either the fibrinogen or the first globulin fraction alone. Another common finding was an increase of both the fibrinogen and first globulin fractions, the other two remaining within normal limits. Less common was an increase of the fibrinogen and second globulin fraction or of the fibrinogen and both globulin fractions. The albumin fraction was never increased, but was not uncommonly reduced either alone or in combination with alteration in the other fractions. Only very rarely was there a reduction of the globulin fractions ; the fibrinogen fraction was never found to be below the lowest normal in disease.

Significance of Viscosity Changes

At an early stage of this investigation it became apparent that the duration of the disease process was of considerable importance in determining the fractional viscosity pattern. If, for example, this is studied during the first week of disease, results are obtained as shown in Table 2.

In all but one instance the fibrinogen fraction is increased, and the second globulin fraction is increased in all instances. The first globulin fraction on the other hand is generally normal, though by the fifth and sixth days in two instances it is beginning to rise above the upper limit of normal. When the fractional viscosity is studied in the second week, the first globulin fraction is found to be raised in most cases ; the fibrinogen is raised as before, but the second globulin fraction is seen to be normal in most instances (Table 3).

In convalescence, on the other hand, the fibrinogen and second globulin fractions are normal, but the first globulin fraction is raised in every instance (Table 4).

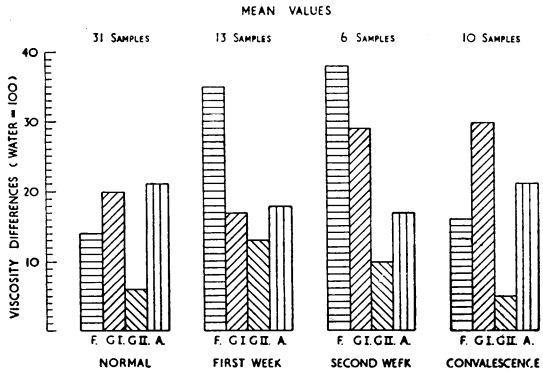

FIG. 2.—Relationship of viscosity pattern to stage of disease.

Fig. 2 shows the mean values for the differential plasma viscosity at each of these stages of disease, and emphasizes the dissociation between the fibrinogen and second globulin fraction on the one hand and the first globulin fraction on the other. This may be further illustrated by serial estimations. For this purpose changes were induced artificially by injecting T.A.B. vaccine, 28 million and then 50 million organisms, intravenously on two consecutive days into a healthy subject. In this way the sequence of events was studied in detail from the

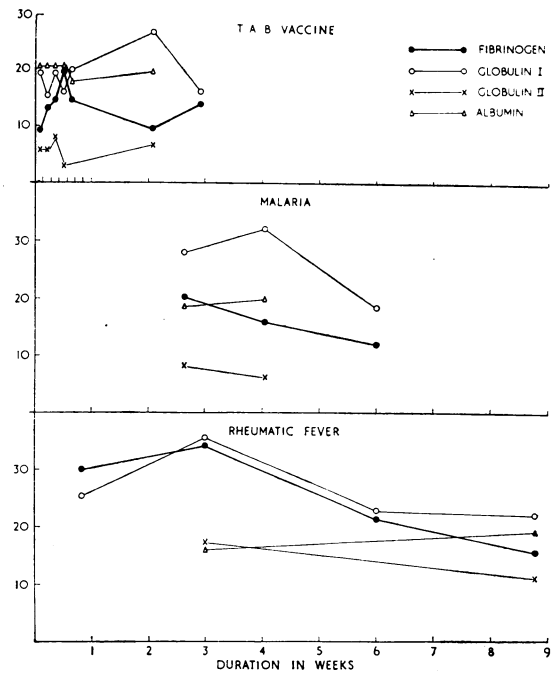

FIG. 3.—Viscosity patterns after T.A.B. vaccine and in malaria and rheumatic fever.

onset of the changes. It will be observed that the fibrinogen starts to increase on the second day, reaches a maximum on the fourth day and then rapidly subsides. The second globulin fraction runs a similar course. The first globulin fraction, on the other hand, remains normal during the first week, then rises in the second week when the others have recovered. The albumin fraction is reduced in the first week and subsequently returns to normal. The sequence suggested by the figures in Tables 2, 3, and 4 is thus confirmed.

Serial estimations have also been carried out in disease, but are necessarily less complete. In estimations during an attack of malaria (Fig. 3) the same sequence can be observed, the first globulin

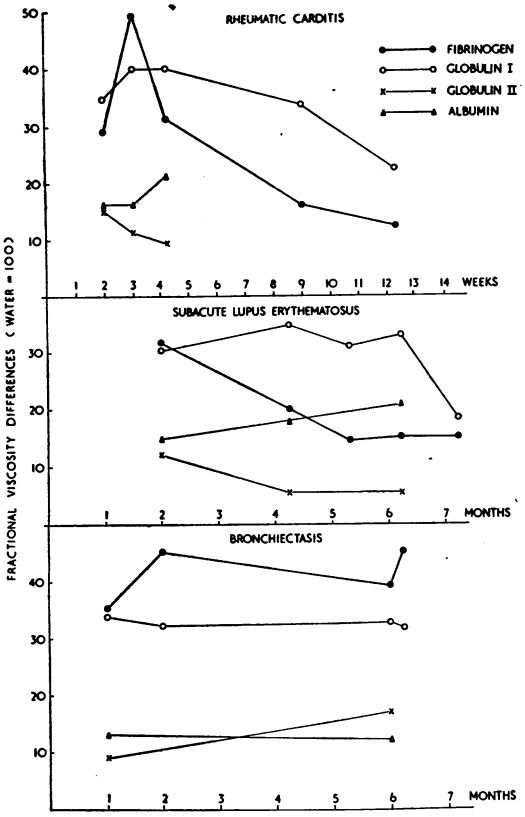

FIG. 4.—Viscosity patterns in rheumatic carditis, subacute lupus erythematosus, and bronchiectasis.

fraction rising as the fibrinogen and second globulin fraction recover. In rheumatic diseases such as rheumatic fever and rheumatoid arthritis, on the other hand, the first globulin fraction tends to be less affected and to subside more rapidly, as shown in the case of rheumatic fever in Fig. 3. Where, however, carditis supervenes, as in the case of rheumatic carditis shown in Fig. 4, a more typical first globulin response is noted, there being a lag of three weeks after the return of the fibrinogen fraction to normal. In subacute disease the changes are similar to those seen in acute cases, and this is well illustrated by the figures from subacute lupus erythematosus (Fig. 4). Here the fibrinogen response is more prolonged, normal figures being obtained only after the fifth month. The second globulin fraction as in acute disease returns to normal shortly before the fibrinogen fraction. The first globulin fraction, on the other hand, does not reach a maximum until the fourth month and then remains raised until the seventh month. The albumin in this case was not substantially reduced, but shows a steady rise synchronously with the recovery of the fibrinogen fraction.

In chronic disease one or two things may happen. There may be a continuous process as in the case of advanced bronchiectasis, shown in Fig. 4, in which the fractions maintain abnormal levels without marked variation, or there may be a recurrent process, as found for example in untreated rheumatoid arthritis or in chronic rheumatic heart disease (Fig. 5). Here the sequence already noted in acute disease recurs in cycles with or without a return to normal figures from time to time.

It should be emphasized that these changes are essentially non-specific. They are found in infections, intoxications, trauma, and ischaemia, in fact in any condition in which there is reason to suppose that tissue damage is taking place. Occasionally, however, viscosity patterns are encountered which may be of some value in diagnosis. In liver disease, for example, the typical finding is a high first globulin viscosity with little or no fibrinogen response (Fig. 6). In advanced cases both the fibrinogen and albumin fractions will be reduced. In rheumatoid arthritis the poor first globulin response has already been noted. In lymph gland disease, as for example Hodgkin's disease, lymphosarcoma, or tuberculous adenitis, the first globulin response may completely fail or this fraction may even be reduced. In renal disease, where there is marked loss of protein in the urine, the albumin and first globulin fractions are most depleted. It should be noted that the albumin and gamma globulin are fractions having the smallest molecules

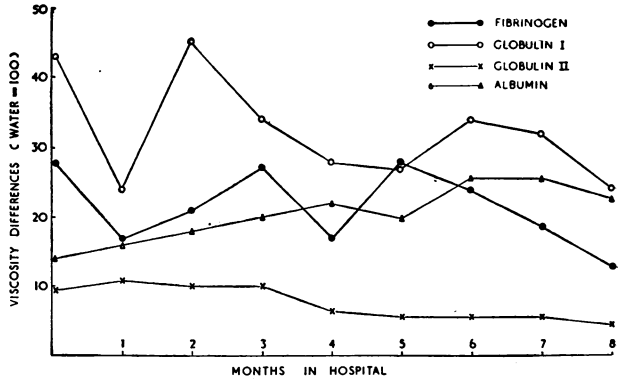

FIG. 5.—Viscosity pattern in chronic disease (remittent type) : rheumatic carditis.

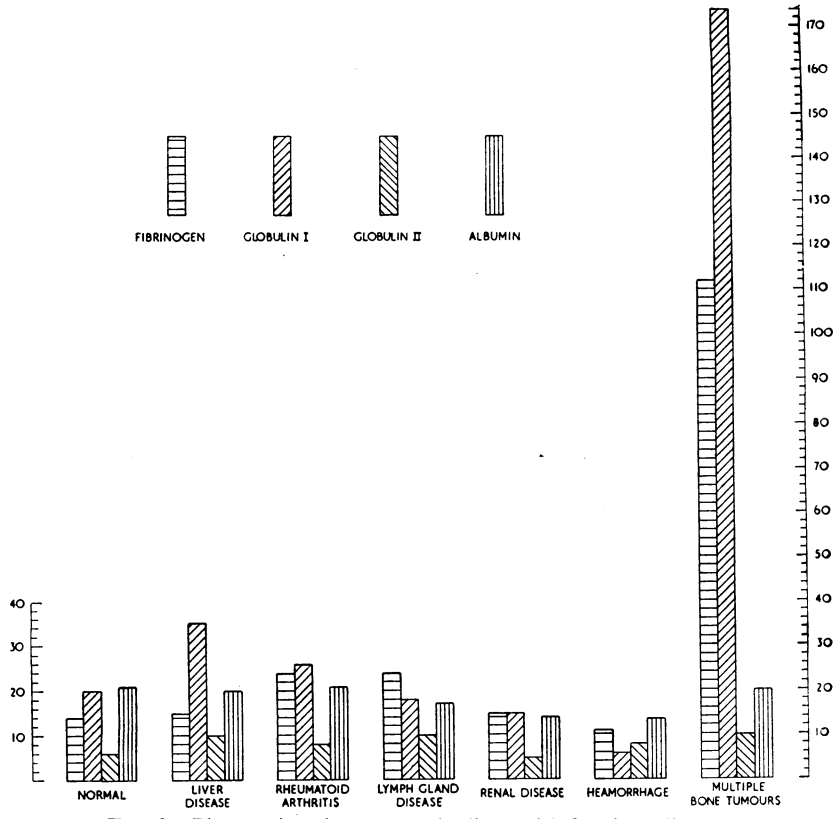

FIG. 6.—Plasma viscosity patterns in diagnosis of various diseases.

and therefore passing more rapidly through a damaged glomerulus. Following haemorrhage all fractions are reduced and the subsequent order of recovery is fibrinogen, globulin II, albumin, and globulin I. The last takes about three weeks to recover, so that it gives most useful information in the later stages of convalescence and is particularly useful when in obscure anaemia the history of haemorrhage is indefinite. On the other hand during the treatment of acute haemorrhage the fibrinogen fraction as it recovers first is most valuable. It is of interest that the changes of the first and second globulin fractions correspond closely with those noted for γ and α globulin respectively in the same diseases by Luetscher (1940 and 1941) and Malmros and Blix (1946). The most typical pattern is encountered in multiple myelomatosis, where the marked increases of fibrinogen and globulin I exceed those encountered in any other disease. Where the first globulin fraction is increased without the second, the growth is presumably a γ-globulin plasmocytoma. In β-globulin plasmocytoma it would be expected that the second globulin fraction would be affected to a relatively greater extent. Similar but less marked changes are encountered in other multiple bone tumours, particularly in carcinomatosis secondary to carcinoma of the prostate gland.

Comparison of E.S.R. and Plasma Viscosity

With regard to the comparative efficiency of the E.S.R. and plasma viscosity as an indication of abnormal changes in disease, a study of 245 cases of clinically active disease gives the following results : E.S.R. abnormal in 43 per cent. ; plasma viscosity abnormal in 59 per cent. ; fractional viscosity abnormal in 86 per cent. ; E.S.R. or fractional viscosity abnormal in 94 per cent.

Allowing for the difficulty in determining clinically the presence of active disease, these figures do indicate that, by the combined use of the blood sedimentation rate and the differential plasma viscosity, abnormality can be detected in a large

proportion of cases. It may be said with certainty that the differential plasma viscosity is never normal in the presence of gross disease, and that where no change is found in either the E.S.R. or differential viscosity any disease present is either very mild or localized. There is one exception to this, namely disease of the central nervous system or of the meninges, in which gross pathology may be present with negligible changes in the blood. In such disease processes, examination of the cerebrospinal fluid gives more reliable information.

Summary

A method is described for studying the factors concerned in the viscosity of the plasma. This method, which has been termed the differential plasma viscosity, provides a simple means of determining the relative changes of the plasma protein fractions, particularly the fibrinogen and γ globulin. A series of plasma samples from healthy individuals and from patients suffering from a wide variety of disease processes were studied by this method.

The results of this study indicate that the changes in disease depend more on the stage than on the nature of the process. During the early stages the fibrinogen and second globulin fraction (presumably α globulin) are increased but the first globulin fraction (γ globulin) remains normal. In the second week the first globulin fraction also increases. In convalescence the first globulin fraction remains raised for a time after the other fractions have returned to normal. The albumin fraction is reduced in the early stages in a proportion of cases. The actual duration of these changes depends on the acuity of the disease. In a transient process the lag between the first globulin fraction on the one hand and the fibrinogen and second globulin fractions on the other, may be a matter only of a week ; in a subacute disorder it may be a month or two. In chronic disorders there may be a constantly altered level of all fractions or a remittent course may be followed. In the latter, the sequence of changes noted in acute disorders may be observed to recur with each relapse.

Following haemorrhage the same sequence may be observed but in reverse. All fractions are at first reduced. The fibrinogen fraction recovers most rapidly, generally within twenty-four hours of cessation of the haemorrhage, and this is followed by the second globulin fraction, the albumin and lastly the first globulin fraction, which may take three weeks to return to normal.

Certain characteristic patterns in disease are noted : the poor globulin I response of rheumatoid arthritis ; the absent response of this fraction in widespread lymph gland disease ; the high globulin I response of liver disease ; and the very high values for both fibrinogen and one of the globulin fractions in multiple tumours of bone, particularly multiple myelomatosis.

Comparison of the differential plasma viscosity with the E.S.R. and the simple plasma viscosity indicates that the first is a more sensitive indicator of pathological plasma protein changes than either of the other two and that the E.S.R. is the least reliable. It is recommended that both the E.S.R. and the differential plasma viscosity be used in doubtful cases.

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Viscosité du Plasma

Résumé

On décrit une méthode pour étudier les facteurs impliqués dans la viscosité du plasma. Cette méthode, qui reçut le nom de viscosité différentielle du plasma, fournit un moyen simple pour déterminer les modifications relatives des fractions protéïniques du plasma, particulièrement du fibrinogène et de la γ globuline. On a étudié par cette méthode une série d'échantillons de plasma des sujets sains et des malades souffrant d'une grande variété de processus morbides.

Les résultats de cette étude indiquent que les changes morbides dépendent plus du stade du processus que de sa nature. Au stade initial le fibrinogène et la seconde fraction de la globuline (probablement la globuline α) se trouvent augmentés, mais la première fraction (globuline γ) demeure normale. Au cours de la deuxième semaine la première fraction de la globuline augmente également. Pendant la convalescence la première fraction de la globuline demeure augmentée pendant un certain temps après que les autres fractions aient récupéré leur valeur normale. La fraction albumine est diminuée pendant la période initiale dans un certain nombre des cas. Dans les processus passagers le décalage entre la première fraction de la globuline d'un coté, et le fibrinogène et la seconde fraction de la globuline de l'autre, peut durer seulement une semaine ; dans un trouble subaigu il peut persister un ou deux mois. Dans les troubles chroniques les valeurs pour toutes les fractions peuvent se trouver

à un niveau modifié ou bien elles peuvent avoir une évolution rémittente. Dans ce dernier cas la succession des changes observés dans les troubles aigus peut se répéter au cours de chaque rechute.

Après une hémorragie on peut voir la même séquence, mais à l'envers. Toutes les fractions se trouvent d'abord diminuées. Le fibrinogène se relève le plus rapidement, généralement endéans de vingt-quatre heures qui suivent l'arrêt de l'hémorragie ; il est suivi de la deuxième fraction de la globuline, de l'albumine et, finalement, de la première fraction de la globuline ; le tout redevient normal au bout de trois semaines.

On note certains types caractéristiques de la maladie : la faible réponse de la globuline I dans l'arthrite rhumatismale ; l'absence de sa réponse dans l'affection étendue des ganglions lymphatiques ; sa forte réponse dan les maladies du foie ; et de très fortes valeurs pour le fibrinogène et pour une des fractions de la globuline dans les tumeurs multiples des os, en particulier dans la myélomatose multiple.

L'étude comparée de la viscosité différentielle du plasma, de la sédimentation globulaire et de la simple viscosité du plasma montre que la première costitue la méthode la plus sensible pour se rendre compte des changes pathologiques de la protéïne du plasma et que la sédimentation globulaire est un indice moins constant. On propose l'emploi de la sédimentation globulaire et de la viscosité différentielle dans les cas douteux.