THE FORMOL-GEL TEST ON PLASMA AND SERUM IN RHEUMATIC CASES

CORRELATION STUDIES AND A SUGGESTION THAT NON-ROULEAUX-FORMING PATHOLOGICAL GLOBULINS MAY BE OF SIGNIFICANCE IN RHEUMATISM

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Introduction

The formol-gel test of Gaté and Papacostas (1920), applied to plasma from cases of rheumatic disease by Gibson and Richardson (1938), has now been the subject of several reports. Some have agreed that the test shows a high degree of correlation with the sedimentation rate (E.S.R.) although it is less sensitive. These include reports by Green *et al.* (1939), Scull and Pemberton (1940), and Milles and Salt (1941). Schultz and Rose (1939) suggested that the test may be an additional aid in determining the presence of carditis in acute rheumatism. Green *et al.* (1939) did not find evidence to support this. It should be noted that while Green used plasma, Schultz and Rose employed serum as in the original Gaté-Papacostas technique. Butterworth and Poindexter (1942) studied serum-gel reactions in acute rheumatism. They could find no evidence of a correlation between the occurrence of positive results and carditis, and they concluded also that the test showed no parallelism with the E.S.R. These discrepancies pointed to a need for further investigation of the test, especially in the relation of plasma-gel formation (P.G.) to serum-gel formation (S.G.) and the correlation of both with the E.S.R.

The fact was early noted by the present writers that correlation between plasma-gel and E.S.R. was just as good, and sometimes better, when "crude" S.R., or S.S. (suspension stability), results were used, as compared with those following correction for anaemia. Gibson and Richardson (1938) noted a close correlation between P.G. tests and anaemia as shown by the packed red cell volume (P.C.V.). Milles and Salt (1941) also noted this and observed that "as the formol-gel (plasma) reactions became more intense the average P.C.V. falls as does the average 'crude' S.S., but the phenomenon is apparently unrelated to the true or corrected S.S." It has been the experience of all who have employed the test that the highest grades of P.G. reaction have been associated constantly with a marked anaemia.

This raises the question of correction of S.S. or E.S.R. readings for anaemia, on which point there is a further marked conflict of opinion among those of wide experience with the sedimentation test. Westergren *et al.* (1931), as a result of plasma protein studies, and in a modified way Kahlmeter (1938), regard correction as unnecessary, as does Cutler (1940) and others. On the other hand, Rourke and Ernstene (1930), Wintrobe and Landsberg (1935), Whitby and Hynes (1938), Collins *et al.* (1939), along with many others, have thought that there is an advantage to be gained by adjustment of the crude rate to a standard red cell count or volume. But if the crude rate correlates better with a demonstrable plasma protein abnormality than the corrected, then the validity of correction is certainly in question.

In this connexion the blood volume studies of Sparks and Haden (1932), Bradley (1938), and especially the extensive work of Robinson (1943), are relevant. These workers all agree in showing that a definite hydraemia may occur in rheumatism. The whole-blood volume of the patients is increased, due to an increase of plasma volume. Robinson has described the "hydraemic syndrome" characterized by loss of flesh, rise in plasma volume, rise in plasma globulins and oligocythaemia which run parallel with one another, but which are not correlated with the E.S.R. He suggests that the increase of plasma volume may be secondary to increase of plasma protein. This would be the reverse process of that seen in renal oedema. As a result of oncotic pressure changes, fluid from the tissues may enter the blood stream. In a statistical study of plasma protein and E.S.R., Fraser and Rennie (1941) showed that plasma protein changes in rheumatism are in the direction of fibrinogen and globulin increase at the expense of albumin. This is in general agreement with many previous studies of the same kind. If the rouleaux-forming proteins giving rise to the increased E.S.R. were the same as those which gave rise to hydraemia, then the case for correction would be weakened. The increase of crude S.R. as a result of hydraemia alone might

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be regarded as an indirect result of tissue destruction in the joints and elsewhere, via plasma protein increase. The sedimentation reaction would then be a composite test, increase of rate being due to two factors, both related to disease activity. Consideration of this point in the light of further evidence was regarded as desirable.

The plasma-gel reaction in rheumatism is probably dependent on the fibrinogen and globulin content of the plasma while the serum-gel is predominantly a globulin effect, if we except the small amount of fibrinogen which is not converted to fibrin and removed with the clot. Wise and Gutman (1937) carried out gel tests on sera in parallel with protein estimations and found that a total protein of 8.5% or more would produce a gel and the test was always positive if the globulin was 4% or more. Sera containing 3 to 4% of globulin were irregularly positive by the gel test, while sera with total protein less than 8% gave positive results if hyperglobulinaemia was present.

The purpose of the present study was, therefore, to carry out gel tests on serum and plasma of bloods from rheumatic cases of various kinds, parallel estimations being made of S.S. and packed cell volumes, the latter being used also for obtaining corrected S.S. values.

Methods

Formol-Gel Tests.—These were carried out with the serum and plasma in the way described by Gibson and Richardson (1938). The same notation for recording intensity of the reaction as shown by the quantitative test was used.

Sedimentation Tests.—The method used throughout is that of Collins *et al.* (1939). The results were checked by a simultaneous estimation in the Wintrobe tube which was later centrifuged for 15 minutes at 3,000 r.p.m. to obtain the P.C.V.

Results

Sedimentation tests to obtain crude and corrected

S.S. readings by the method of Collins *et al.* (1939) were carried out in parallel with plasma-gel (P.G.), serum-gel (S.G.), and haematocrit (P.C.V.) estimations in a series of consecutive rheumatic cases of all clinical types.

The first point to be determined was the relationship which the gel tests showed to one another. In Table 1, tests of P.G. and S.G. (both quantitative) are recorded on 582 bloods. The figures given show that the plasma-gel test is never negative when the serum-gel is positive and that the serum-gel reaction, when positive, is always weaker than the plasma-gel on the same blood. The suggestion is that the P.G. effect includes the S.G. factor plus an additional factor. The dilution showing a positive serum-gel is always at least 2 grades below the plasma reading and the S.G. reaction is frequently much less intense. Thus no blood gave complete solidification of serum (3+) when the corresponding plasma did not give complete solidification in dilution 0.8 in 1 (5+). Serum reactions of greater intensity than 3+ were exceedingly rare (21 out of 582 bloods tested, or 3.6%). Only in bloods of plasma-gel 8+ or 9+ (0.5 or 0.4 in 1 dilution) were all serum-gel tests positive. The total of P.G. positive results in this heterogeneous series amounted to 56% and of S.G. 12.5%. Thus formol-gel tests in any disease would be expected to give widely divergent results according to whether plasma or serum has been used. The table also shows that it is not possible to estimate what the serum-gel result would be from a knowledge of the plasma-gel unless the latter were very intense (8+ or 9+). Conversely, from a positive serum-gel it would only be possible to assume that the plasma test would also be positive and of greater intensity. Thus from Table 1 it will be seen that a 3+ S.G. reaction may be associated with 5+, 6+, 7+, 8+, or 9+ in the plasma. From the figures given by Wise and Gutman (1937) it will be seen that in certain cases of rheumatism there is a very marked hyperglobulinaemia, since gel formation occurs in dilutions up to 60%.

TABLE 1
PLASMA-GEL AND SERUM-GEL RESULTS IN 582 CONSECUTIVE CASES OF RHEUMATISM. NUMBER OF CASES IN EACH GROUP

Serum-Gel	Plasma-Gel										Total
	0	1+	2+	3+	4+	5+	6+	7+	8+	9+	
0	224	25	25	58	74	89	12	2			509
1+			1	1	2	7	4	2			17
2+			1	2	1	1	3	3			11
3+						9	12	1	1	1	24
4+							8	6			14
5+								1	3		4
6+									1	1	2
7+										1	1
Total ..	224	25	27	61	77	106	39	15	5	3	582
Serum-gel %+	0	0	7	5	4	16	70	87	100	100	

CORRELATION OF GEL REACTIONS WITH E.S.R.

Tables 2 and 3 show the number of positive plasma and serum tests in each S.R. group in 480 cases. In Table 2 the crude S.S. is used. The results for P.G. closely parallel our previous figures (Gibson and Richardson, 1938). The vast majority

TABLE 2.

CRUDE S.S. PLASMA-GEL AND SERUM-GEL TESTS ON 480 SAMPLES OF BLOOD FROM RHEUMATIC CASES

Crude S.S.	P.G.— S.G.—		P.G.+		S.G.+		Total
	No.	%	No.	%	No.	%	
—100	74	98.7	1	1.3	—	0	75
— 89	46	92	4	8	—	0	50
— 79	29	5	24	45	2	3	53
— 69	10	8	103	91	5	4	113
< 59	7	4	182	96	52	27.5	189
	166		314		59		480

TABLE 3.

CORRECTED S.S. PLASMA-GEL AND SERUM-GEL TESTS ON 480 SAMPLES OF BLOOD FROM RHEUMATIC CASES

Corrected S.S.	P.G.— S.G.—		P.G.+		S.G.+		Total
	No.	%	No.	%	No.	%	
—100	39	95	2	5	—	0	41
— 89	78	95	4	5	—	0	82
— 79	29	43	39	57	5	7	68
— 69	20	9	191	91	31	15	211
< 59	—	0	78	100	23	29	78
	166		314		59		480

of bloods with S.S. under 70% gave positive reactions. In the S.S. group 70–79, approximately half were positive, and above S.S. 80% the proportion of positive reactions was very small.

The incidence of positive S.G. reactions was much lower, and even in the most rapid S.R. group the proportion of positive results was only 27.5%. It may be said that the most rapid sedimentation rates are associated with a negative serum-gel test in a high proportion of cases.

In Table 3 the corrected S.S. is correlated with the gel tests. Correction eliminates all negative P.G. results from the lowest S.S. group, but increases the number of positive P.G. cases in the normal S.S. group. Otherwise the results are not strikingly different as compared with the crude S.S. findings in Table 2.

GROUPING OF CASES ACCORDING TO S.S., P.G. AND S.G. RESULTS

From long experience of this method of performing the blood sedimentation test in normal in-

dividuals and in medical and surgical cases of all kinds, we are inclined to regard 90% (corrected S.S.) as the strict normal. On this criterion, the cases may be divided into four groups by the use of E.S.R. and Gel tests (see Table 4).

TABLE 4.

GROUPING OF RHEUMATIC CASES ACCORDING TO THE RESULTS OF E.S.R., PLASMA-GEL AND SERUM-GEL RESULTS: 480 CASES

Group	E.S.R.	P.G.	S.G.	No. of Cases	Percentage of Total
1	Not increased	—	—	41	9
2	Increased	—	—	127	26
3	„	+	—	253	53
4	„	+	+	59	12
Total	..	..	..	480	100

It will be seen that the very occasional aberrant cases (two are shown in Table 3) with P.G. positive and normal corrected S.S. are omitted. They are probably explained by the lag in development or return to normal of P.G. as compared with S.S. as shown clearly by Green *et al.* (1939).

The abnormal bloods may be classified as being in what we propose to call the early fibrinogen phase (S.R. increase only), the late fibrinogen phase (S.R. increase: P.G.+) or the globulin phase in which all three tests are positive. Group 1, without E.S.R. or gel abnormality, includes the fibrositic cases and most, but not all, of the osteo-arthritis. The remainder of the osteo-arthritis cases occur in Group 2. Groups 2 and 3 contain a majority of the rheumatoid arthritis and spondylitis cases. Group 4 (globulin phase) is associated with the most severe forms of rheumatoid arthritis, especially in young people, in whom muscle wasting and anaemia are extreme. Taussig (1938) noted an association between Still's Disease and hyperglobulinaemia, and extended that diagnosis to include cases in adults which show marked emaciation, splenomegaly, adenopathy, anaemia, and pericarditis. Robinson (1943) has mentioned loss of flesh and anaemia as being cardinal signs of the "hydraemic syndrome." The association between hyperglobulinaemia, anaemia, and emaciation is therefore well recognized.

Chronicity appears to influence the nature of the plasma protein changes. Berger (1922) and Westergren *et al.* (1931) have found that fibrinogen increase tends to be associated with acute conditions, globulin increase with chronic. It is of interest in this connexion that we (Gibson and Richardson, 1938, and Gibson and Kersley, 1938) have noted that acute gout cases tend to be allocated to what we now describe as the "early fibrinogen phase." It may be that in these cases sufficient time has not elapsed for the development of the plasma-gel reaction. The three phases suggested may be a measure of chronicity, of severity, or possibly of a qualitative difference among the cases.

GEL REACTION AND HAEMATOCRIT READINGS

In Table 5 are shown the results of haematocrit determinations (P.C.V.) correlated with P.G. and S.G. tests on 480 patients. The P.G. positive incidence rises with a falling P.C.V. No positives were found in blood of P.C.V. 50% or over. 27% show a positive P.G. at P.C.V. 45-49, a group of patients who are free from anaemia. With diminishing P.C.V. the proportions of positive P.G. results rises nearly to 100%.

TABLE 5.
P.C.V. (HAEMATOCRIT READING) CORRELATED WITH
PLASMA-GEL AND SERUM-GEL TESTS

P.C.V.	P.G.— S.G.—		P.G. +		S.G. +		Total
	No.	%	No.	%	No.	%	
50+	22	100	—	0	—	0	22
45-49	59	73	22	27	—	0	81
40-44	67	37	115	63	13	7	182
35-39	15	10	131	90	29	20	146
<35	3	6	46	94	17	35	49
	166		314		59		480

As would be expected from the previous tables, the S.G. positive results are fewer. None occur in bloods with P.C.V. over 45%. The proportion of positive results increases inversely with the P.C.V., until in the most anaemic group it is 35%.

The association of serum-gel reactions with anaemia was further analysed in Table 6, which is constructed from observations on 278 bloods of low S.S. In so far as rouleaux-forming constituents

TABLE 6.
HAEMATOCRIT AND FORMOL-GEL TESTS

Showing correlation between (1) Plasma-Gel and P.C.V., and (2) Serum-Gel and P.C.V. in a group of bloods, all of which showed a marked increase in E.S.R.
Total 278 bloods with Crude S.S.<70%

Haemato- crit	Total	P.G. +		S.G. +	
		No.	%	No.	%
45-49	8	8	100	—	0
40-44	101	94	94	11	11
35-39	123	117	95	25	20
<35	46	44	96	16	35

are concerned, the group is fairly homogeneous. While the plasma-gel results are almost constant throughout the range, the serum-gel incidence shows an increase of positive reactions with the P.C.V. The positive serum-gel reactions were also found to be more intense in the lowest haematocrit group. The average S.G. reaction was 3.8+ in P.C.V. group 35% compared with 2.8+ in P.C.V. group 40-44%. This inverse correlation of P.C.V.

and globulin increase at once recalls the findings of Robinson (1943) on hydraemia in rheumatism. The suggestion is that the low haematocrit readings are related to the serum proteins and that the serum-gel reaction may be an indication, though possibly an insensitive one, of these protein changes. It is also suggested that the serum-gel results are to a considerable extent unrelated to the E.S.R., though not entirely so. This is in keeping with the work of Coburn and Kapp (1936), Fraser and Rennie (1941), and Westergren *et al.* (1931), who found the best correlation to exist between E.S.R. and fibrinogen while rise of globulin had only a slight influence in E.S.R.; the failure of Butterworth and Poindexter (1942) to get any significant correlation between E.S.R. and serum-gel is also in agreement. The presence of an increase of non-rouleaux-forming globulin would also explain the finding of Robinson (1943) that the essential E.S.R. was not correlated with the increase of plasma volume in rheumatism, and that of Milles and Salt on the apparent relation between anaemia and the intensity of the plasma-gel test.

It is not suggested that this is applicable to all diseases in which E.S.R. is increased. Bendien and Snapper (1931), and Gilligan and Ernstene (1934), have made the interesting observation that in certain cases of liver disease the globulins appear to be the main site of the rouleaux-forming effect rather than fibrinogen. This state of affairs, however, appears to be exceptional. Conversely, the results of Mester (1945) give the suggestion that in fibrositis an increase of non-rouleaux-forming fibrinogen may be present unassociated with any hydraemic tendency. Our observations, taken with those of others in this field, could be best explained by the hypothesis that in certain cases of rheumatism two groups of plasma protein abnormalities may be present.

1. Increase of rouleaux-forming proteins, responsible for the increase of E.S.R. and closely associated with the plasma-gel reaction. The rouleaux-forming effect resides mainly in the fibrinogen and to a less extent in the globulin.

2. Increase of non-rouleaux-forming proteins, mainly a globulin increase. This is not correlated with the E.S.R. While all plasma proteins may contribute to the production of hydraemia, the evidence quoted above suggests that the globulins are of major importance.

Whether these effects are due to new pathological proteins or alterations in the physical state of normal proteins is a question which cannot be finally answered.

It may well be that in all cases of active rheumatoid arthritis and spondylitis there is some increase of non-rouleaux-forming globulins, too slight to be detected by the coarse measurement of the serum-gel test. That such changes may occur is suggested by such reactions as the Vernes Resorcinol test and the differential serum sedimentation test of Coke (1937), in both of which serum is employed and which are thus largely independent of fibrinogen effects. To assess the whole plasma picture in

rheumatism it may be necessary to carry out two groups of tests, the E.S.R. and another to estimate the non-rouleaux-forming proteins. Taussig's work, already quoted, on the possibility of a type of rheumatoid arthritis with unusual clinical features and a marked hyperglobulinaemia, suggests that there may be other useful clinical correlations in this largely neglected field of plasma study in rheumatism.

Discussion

If the hypothesis be accepted that there may be an increase of non-rouleaux-forming globulin as well as that of rouleaux-forming protein in diseases such as rheumatoid arthritis which are associated with a rapid E.S.R., then many obscure points in recent work would be elucidated. The hydraemia which has been reported by plasma volume studies and which is suggested by low haematocrit readings may be attributable in part at least to the amount of this non-rouleaux-forming protein. It would explain the failure of many workers (e.g. Aldred-Brown and Munro, 1934) to show a really satisfactory correlation through a wide range, between plasma protein estimations and E.S.R. The literature on this point is reviewed by Davison (1940), who also failed to show any complete correlation.

Whether or not correction for anaemia is necessary appears to depend on what information the observer expects to obtain from the test. If he wishes to form a quantitative estimate of the rouleaux-forming effect of the plasma, then correction is, in our opinion, necessary. A subsidiary advantage is that correction, by most methods in common use, involves the use of the haematocrit, which is our only routine measure, albeit an indirect one, giving some indication of the presence or absence of hydraemia. It is in itself an independent yardstick of progress in rheumatism. Again it is not possible to say in a given case how far an anaemia is real, i.e. the result of toxic destruction or inhibition of production of red cells, and how far a dilution effect. It is an interesting question how far the low haemoglobin and P.C.V. of rheumatism, and indeed in many other chronic diseases, is in fact evidence of a "true anaemia." Only studies of whole-blood volume can give a final answer to this question.

Our own method of reporting E.S.R. results is to chart the curve of the crude S.S. over two hours and to report on the same chart any previous curve, so that the clinician can see at a glance the evidence of progress or deterioration shown by the blood complex. If the curves differ markedly, he can refer to and compare the haematocrit readings, and finally compare the corrected S.S. figures. In this way he can assess to what extent any difference between the curves can be attributed to red-cell

volume changes alone. Our opinion is that the crude S.S. or S.R. has a value, being the resultant of various factors—namely, rouleaux-formation, hydraemia, and true anaemia. The haematocrit and corrected S.S. figures provide a means of analysis without which much of the value might be lost.

Summary and Conclusions

1. Correlation studies have been made between formol-gel tests made on plasma and serum in the same patient.

2. Plasma-gel and serum-gel tests have been further correlated with E.S.R. and packed red-cell volume.

3. The serum-gel reaction is much weaker and the incidence of positives is much lower than the plasma-gel reaction.

4. By the use of S.S., P.G., and S.G., abnormal bloods in rheumatism can be classified into three groups, probably corresponding to degrees of chronicity and severity.

5. In bloods of rapid E.S.R. (low S.S.) the serum-gel test shows a better correlation with the P.C.V. than does the plasma-gel.

6. It is suggested that an increase of non-rouleaux-forming globulins may be present in pathological blood, and that these may be concerned with the production of hydraemia.

7. The bearing of this on the validity of correction of E.S.R. for anaemia is discussed.

REFERENCES

- Aldred-Brown, G. R. P., and Munro, J. M. H. (1934). *Lancet*, **1**, 1333.
 Bendein, W. M., and Snapper, I. (1931). *Biochem. Z.*, **235**, 14.
 Berger, W. (1922). *Z. ges. exper. Med.*, **28**, 1.
 Bradley, W. H. (1938). *Proc. Oxford Congress on Rheumat.*, London, p. 86.
 Butterworth, J. S., and Poindexter, C. A. (1942). *Amer. J. med. Sci.*, **203**, 278.
 Coburn, A. F., and Kapp, E. M. (1936). *J. Clin. Invest.*, **15**, 715.
 Coke, H. (1937). *Charterhouse Rheum. Clinic Orig. Papers*, Oxford University Press, **1**, 37. London.
 Collins, D. H., Gibson, H. J., Race, J., and Salt, H. B. (1939). *Annals of the Rheumatic Diseases*, **1**, 333.
 Cutler, J. W. (1940). *J. Lab. clin. Med.*, **26**, 542.
 Davison, R. (1940). *Ibid.*, **25**, 935.
 Fraser, T. N., and Rennie, J. B. (1941). *Brit. J. exp. Path.*, **22**, 81.
 Gaté and Papacostas (1920). *C. R. Soc. Biol., Paris*, **83**, 1432.
 Gibson, H. J., and Richardson, E. W. (1938). *Acta. Rheum. Amst.*, **10**, 30.
 —, and Kersley, G. D. (1938). *Med. Pr.*, **196**, 353.
 Gilligan, D. R., and Ernstone, A. C. (1934). *Amer. J. med. Sci.*, **187**, 552.
 Green, C. A., Thomson, S., and Glazebrook, A. J. (1939). *Annals of the Rheumatic Diseases*, **1**, 180.
 Kahlmeter, G. (1938). In *A Survey of Chronic Rheumatic Diseases*, London, p. 89.
 Mester, A. J. (1945). *Annals of the Rheumatic Diseases*, **4**, 57.
 Milles, L., and Salt, H. B. (1941). *Ibid.*, **2**, 192.
 Robinson, G. L. (1943). *Ibid.*, **3**, 207.
 Rourke, M. D., and Ernstone, A. C. (1930). *J. Clin. Invest.*, **8**, 545.
 Schultz, M. P., and Rose, E. J. (1939). *Publ. Hlth. Rep. Wash.*, **54**, 248.
 Scull, C. W., and Pemberton, R. (1940). *Ann. intern. Med.*, **14**, 993.
 Sparks, M. I., and Haden, R. L. (1932). *Amer. J. med. Sci.*, **184**, 753.
 Taussig, A. E. (1938). *J. Lab. clin. Med.*, **23**, 833.
 Westergren, A., Theorell, H., and Widström, G. (1931). *Z. ges. exper. Med.*, **75**, 668.
 Whitby, L. E. H., and Hynes, M. (1938). *Lancet*, **2**, 249.
 Wintrobe, M. M., and Landsberg, J. W. (1935). *Amer. J. med. Sci.*, **189**, 102.
 Wise, C. R., and Gutman, A. B. (1937). *Ibid.*, **194**, 263.
 Woods, J. W. Jr., and Comroe, B. I. (1945). *J. Amer. med. Ass.*, **127**, 582.