PLASMA ANTIHEPARIN ACTIVITY AND THE HEPARIN PRECIPITABLE FRACTION OF PLASMA IN RHEUMATOID ARTHRITIS

BY

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An increase in the antiheparin activity of the plasma occurs in conditions in which there is an increased tendency to thrombosis. Thus an increase in antiheparin activity occurs in the week or two after surgery (Godal and Fichera, 1961; Holger-Madsen and Schiøler, 1959; Gormsen and Haxholdt, 1960), after myocardial infarction (Beaumont, Chevalier, and Lenegre, 1948; Holger-Madsen, 1960), and after trauma (Innes and Sevvitt, 1964). The mechanism underlying this phenomenon is not known but it is generally accepted to reflect increased coagulability.

There is little information in the literature to indicate whether there is an increase in heparin activity or whether there is antiheparin activity in the blood of patients with rheumatoid arthritis. Antiheparin activity was found in seven patients with rheumatoid arthritis by Bally (1951), and Lörincz and Sas (1966) found that in rats with adjuvant arthritis the serum heparin level becomes labile and increases in some cases. In preliminary studies which we carried out on patients with rheumatoid arthritis we found that plasma antiheparin activity appeared to be increased. We therefore investigated antiheparin activity in a larger number of patients and compared the results with those obtained in a control group of healthy subjects matched for age and sex. Measurements were also made of serum protein levels, plasma fibrinogen concentration and the heparin precipitable fraction of the plasma to find out whether there was any correlation between antiheparin activity and any of these parameters.

Material

The subjects investigated comprised 34 adult hospital patients with classical or definite rheumatoid arthritis (A.R.A. criteria: Ropes, Bennett, Cobb, Jacox, and Jessar, 1959) of at least 6 months' duration and with an ESR (Westergren, 1921) of more than 20 mm. in the first

hour. There were seventeen men (mean age 53.1 years) and seventeen women (mean age 55.8 years). The mean duration of the disease was 6.8 years in the men and 9.4 years in the women. The seventeen men and seventeen women studied as controls were apparently healthy I.C.I. employees or healthy subjects referred by the Geriatric Unit at Crumpsall Hospital. The mean age of the men was 54.2 years and of the women 55.1 years. Only 25 of these controls (fourteen men and eleven women) were used in the comparison of the values of the serum albumin and globulin fractions in the patients and normal subjects; their mean age was 53.6 years for the men and 56.3 years for the women.

Methods

Blood samples were taken from the patients during the first week after admission to hospital and a further sample a week later. The mean values obtained for each of the parameters measured in these two samples were used in the statistical analysis of the results. Further blood samples were taken 4, 6, and 8 weeks after admission. Two blood samples were taken from the control subjects with an interval of one week between each sample and the mean values were used for analysis.

Plasma Antiheparin Activity.—This was assayed by measuring the heparin-thrombin clotting time using a range of concentrations of heparin. The technique was similar to that previously described (Cotton and Wade, 1964). The principle of the method is to add heparin to platelet-poor citrated plasma, allow the mixture to incubate for 3 min. at 37° C., and then to note the thrombin clotting times. Heparin solutions containing 25, 35, 45, 55, 65, 75, and 100 i.u./100 ml. are used, the lowest heparin concentration is noted at which there is failure of the plasma to clot within 20 minutes. (Clotting times of over 20 minutes are taken as being infinite as the fibrin clots then formed are too fine for the end point to be measured accurately.)

Plasma Fibrinogen Concentration.—The method of Lempert (1962) was used.

Heparin Precipitable Fraction of Plasma.—The technique used was similar to that described by Smith (1957). The method was modified by adding 4.5 ml. blood to 0.5 ml. saline containing 1 mg. heparin. After the precipitate formed at 4° C. had been washed three times with cold phosphate buffer (0.05 M; pH 7.4), the precipitate was dissolved in 1.5 ml. of this buffer at 37° C. The tyrosine content was then assessed using Folin-Ciocalteu reagent (Lempert, 1962).

Serum Proteins.—The method of Briere and Mull (1964) was used.

Results

The results obtained from the measurement of antiheparin activity in the rheumatoid patients and healthy controls are illustrated in Fig. 1. The percentage of subjects in each group whose clotting time reaches "infinity" at the concentration of heparin indicated is shown. Thus, using a concentration of 35 i.u./100 ml. in the clotting system, 70 per cent. of the healthy control subjects have clotting times greater than 20 minutes, whereas only 23 per

Fig. 1.—Antiheparin activity. Relation between percentage of patients with a clotting time of infinity and concentration of heparin used in the clotting system.

cent. of the patients had a similar prolongation of clotting time. A statistical analysis of these results is shown in Table I. Using the clotting systems containing the 35 and 45 i.u. heparin/100 ml., a significant increase in heparin resistance is demonstrated in the patients with rheumatoid arthritis as compared with the healthy controls. These results are in accord with those obtained by Bally (1951),

who was able to demonstrate an increase in heparin resistance *in vitro* and *in vivo* in seven patients with rheumatoid arthritis. The samples taken at 4, 6, and 8 weeks after admission to hospital showed no significant difference in antiheparin activity as compared with those taken in the first two weeks.

TABLE I
SIGNIFICANCE OF DIFFERENCE IN ANTIHEPARIN ACTIVITY BETWEEN THE PATIENTS WITH RHEUMATOID ARTHRITIS AND HEALTHY CONTROLS AS ASSESSED USING THE TWO STRENGTHS OF HEPARIN WHICH APPEARED TO BE OPTIMAL FOR THIS STUDY

Heparin Concentration (i.u./100 ml.)	χ^2	P*
35	11.580	< 0.0005
45	7.719	< 0.005

*The P values are for single-sided significance levels

The results obtained by the measurement of plasma fibrinogen and other proteins are shown in Table II. Apart from beta globulin and total serum protein, there are significant differences in the mean values of the proteins in the patients with rheumatoid arthritis as compared with the control subjects. There are significant increases in plasma fibrinogen, heparin precipitable fraction of plasma (HPF), alpha₁, alpha₂, and gamma globulin, and a decrease in the serum albumin level. The mean ratio of HPF to fibrinogen in the control subjects was 0.256 and in the patients 0.365. An increase in plasma fibrinogen concentration in rheumatoid arthritis has been observed by Fearnley, Chakrabarti, and Hocking (1965) and an increase in HPF in this condition has been noted by Smith (1957) and Thomas, Smith, and von Korff (1954). In the patients studied here there was a significant correlation between HPF and fibrinogen ($r = 0.710$; $P < 0.001$) as well as in the healthy control subjects ($r = 0.583$; $P < 0.01$). There were individual fluctuations in fibrinogen, HPF, and the serum protein levels in the blood samples taken at the 4th, 6th, and 8th week after admission, but the mean values did not differ significantly from the values obtained during the first fortnight.

Multiple linear regression was used to ascertain whether a linear relationship existed between antiheparin activity, and the parameters listed in Table II (opposite) in the patients with rheumatoid arthritis. The only variable showing a linear relation with antiheparin activity using this method of analysis was HPF.

TABLE II
MEAN VALUES, STANDARD ERRORS OF THE MEANS, AND THE SIGNIFICANCE OF DIFFERENCE BETWEEN THE MEANS FOR EACH PARAMETER STUDIED IN THE TWO GROUPS OF SUBJECTS INVESTIGATED

Parameter (mg. per cent.)	Series		Significance of Difference between Means
	Rheumatoid Arthritis	Normal Subjects	
Plasma Fibrinogen	553.6 ± 31.66 (34)	394.6 ± 18.7 (34)	<i>t</i> = 4.325 P < 0.001
Heparin Precipitable Fraction of Plasma ..	202.3 ± 21.20 (34)	101.2 ± 11.03 (34)	<i>t</i> = 4.231 P < 0.001
Albumin	3.42 ± 0.067 (34)	4.03 ± 0.078 (25)	<i>t</i> = 5.848 P < 0.001
α ₁ Globulin	0.419 ± 0.005 (34)	0.328 ± 0.014 (25)	<i>t</i> = 4.381 P < 0.001
α ₂ Globulin	1.25 ± 0.0385 (34)	0.956 ± 0.027 (25)	<i>t</i> = 5.805 P < 0.001
β Globulin	1.14 ± 0.037 (34)	1.031 ± 0.039 (25)	<i>t</i> = 2.015 P < 0.1
γ Globulin	1.39 ± 0.0848 (34)	1.091 ± 0.053 (25)	<i>t</i> = 2.727 P < 0.02
Total Protein	7.37 ± 0.103 (34)	7.39 ± 0.112 (25)	<i>t</i> = 0.129 P < 0.9

The figures in brackets indicate the number of measurements made in each group.

The results obtained from correlating each variable with increasing antiheparin activity are shown in Table III. Antiheparin activity was taken as the dependent variable and the other parameters as independent variables. It is seen that an increase in heparin resistance over the range of solutions of 35 to 45 i.u. heparin/100 ml. is correlated with a decrease in alpha₁ globulin (P < 0.05) and alpha₂ globulin (P < 0.02). An increase in heparin resistance over the range of heparin solutions of 45 to 55 i.u./100 ml. is correlated with a highly significant increase in HPF (P < 0.001); an increase in fibrinogen (P < 0.01); a decrease in albumin (P < 0.01) and an increase in alpha₁ globulin (P < 0.01).

HPF and that this is the only variable which has a statistically significant linear relationship with antiheparin activity. The other variables which were observed to correlate with antiheparin activity do so in different directions according to the concentration of heparin in the system. At the lower dilutions of heparin, increasing antiheparin activity is correlated with a reduction in the variable, and at higher concentrations of heparin, increasing antiheparin activity is associated with an increase in the variable. Over the range of heparin concentrations used therefore (35 to 55 i.u./100 ml.), the effects of these other variables on antiheparin activity are cancelled out and HPF is left as the only variable having a significant linear relationship with antiheparin activity.

TABLE III
‘*t*’ VALUES AND P VALUES OBTAINED BY CORRELATING SERUM PROTEINS, FIBRINOGEN, AND HEPARIN PRECIPITABLE FRACTION OF FIBRINOGEN WITH HEPARIN RESISTANCE

Increase in Heparin Resistance	35 units to 45 units		45 units to 55 units	
	‘ <i>t</i> ’ value	P	‘ <i>t</i> ’ value	P
Fibrinogen	-1.50	> 0.1	+3.19	< 0.01
HPF	+0.52	> 0.5	+4.69	< 0.001
Albumin	+1.58	> 0.1	-2.85	< 0.01
Albumin/Globulin	+0.24	> 0.5	-1.49	> 0.1
α ₁	-2.24	< 0.05	+3.30	< 0.01
α ₂	-2.67	< 0.02	+1.74	> 0.1
β	-1.15	> 0.3	+1.94	> 0.1
γ	+0.39	> 0.5	-0.92	> 0.3

Discussion

In this group of patients with rheumatoid arthritis there were significant increases in plasma antiheparin activity, plasma fibrinogen concentrations, and HPF levels. Of the various parameters studied, HPF was the only one to show a significant linear correlation with antiheparin activity.

The heparin precipitable fraction of human plasma has been investigated by Smith and von Korff (1957) and was found closely to resemble fibrinogen. Shainoff and Page (1960) suggested that HPF is a fibrinogen intermediate formed as the result of the limited action of thrombin on fibrinogen *in vivo* with consequent loss of some of its highly charged fibrinopeptide. Increased amounts of HPF have

It would appear, therefore, that the parameter which correlates best with antiheparin activity is

been reported in rheumatic fever, rheumatoid arthritis, various neoplastic diseases, and pregnancy (Smith, 1957); in post-operative patients (Godal, 1962); and in patients with thromboangiitis obliterans and in atherosclerotic obliterative disease (Craven and Cotton, 1967). HPF also appears in the plasma of rabbits treated with intravenous endotoxin (Thomas, Smith, and von Korff, 1955) or intravenous thrombin (Lee, 1962). It has been suggested by Thomas and others (1955) that HPF may be a precursor of the fibrinoid-like material deposited in the generalized Shwartzman reaction. In the patients and control subjects studied here there was a significant correlation between HPF and fibrinogen but no such correlation was noted in patients with atherosclerotic obliterative disease (Craven and Cotton, 1967; Cotton, Shaikh, and Dent, 1968).

In patients with rheumatoid arthritis we found that there was a significant correlation between the plasma levels of HPF and cryoprecipitate obtained from citrated plasma. Cryoprecipitate was obtained by thawing frozen citrated plasma at 4° C. for 24 hours, and is probably similar to the cryoprecipitate of Pool and Shannon (1965) which is obtained using acid-citrate dextrose as anticoagulant. The protein content of this precipitate and that of HPF from heparinized plasma, obtained at the same time from the patients, was estimated by measuring the tyrosine content. The results obtained in 28 patients are summarized in Fig. 2. It is seen that there is a high correlation ($r = 0.912$; $P < 0.001$) between the levels of HPF and cryoprecipitate. It was found that the fibrinogen content of the cryoprecipitate was less than that of HPF; the cryoprecipitate contained approximately 40 to 50 per cent. clottable protein, whereas HPF contained approximately 85 per cent. clottable protein. It would appear that the presence of optimal amounts of heparin produces a more selective precipitation of this fraction of fibrinogen than cold alone.

If there is a cause and effect relationship between HPF and antiheparin activity, then it is possible that heparin could form a complex with this fraction of fibrinogen so that there is not so much heparin available to interfere with coagulation, and this may be the mechanism producing increased antiheparin activity in rheumatoid subjects as measured here. Antiheparin activity is not merely a phenomenon *in vitro* in rheumatoid subjects, for it has been demonstrated *in vivo* by Bally (1951), who found that the administration of a standard dose of heparin to patients with rheumatoid arthritis did not prolong the blood clotting time to the same degree as that obtained in control subjects.

Fig. 2.—Correlation between cryoprecipitate (obtained by thawing citrated plasma at 4° C. for 24 hours) and heparin precipitable fraction of the plasma in 28 patients with rheumatoid arthritis. The calculated regression line and the 95 per cent. confidence limits are shown.

The interpretation of the possible clinical significance of these observations in rheumatoid arthritis is speculative. The changes which occur in the plasma are those which are generally taken to reflect an increased clotting tendency, *i.e.* an increase in plasma fibrinogen concentration; an increase in a relatively insoluble fibrin intermediate; and an increase in antiheparin activity. Furthermore, it is known that fibrinolysis is impaired in these patients (Chakrabarti, Fearnley, and Hocking, 1964). On the other hand, the incidence of large vessel thrombosis is relatively uncommon as compared with hospital patients with other medical conditions who have a similar degree of physical disability (Ball, 1968). One can only postulate that there must be some protective mechanism against intravascular clotting which has not so far been detected. Alternatively, it is possible that procoagulant material which is forming in the circulation, *i.e.* fibrinogen-fibrin intermediates, is being more efficiently removed by the heightened phagocytic potential of the hyperplastic reticulo-endothelial system found in rheumatoid arthritis (Richmond, Roy, Gardner, Alexander, and Duthie, 1958; Gardner, 1965). The existence of such a mechanism for the removal of circulating fibrin has been proposed by Lee (1962). It has been shown (Beeson, 1947) that rabbits become more

responsive to the development of the Schwartzman reaction after reticulo-endothelial blockade by thorotrast. It is also possible to produce the Schwartzman reaction with a single injection of endotoxin in cortisone-treated rabbits, probably through blockade of the reticulo-endothelial system by cortisone (Thomas and Good, 1952).

It is conceivable, therefore, that there are conditions in which the potential of the reticulo-endothelial system for removing circulating fibrin products is increased and that such a situation occurs in rheumatoid arthritis.

Summary

In an investigation of certain haematological changes in 34 patients with rheumatoid arthritis, a significant increase in plasma antiheparin activity was noted as compared with control subjects matched for age and sex. There were also significant increases in plasma fibrinogen concentrations and

in the levels of the heparin precipitable fraction of fibrinogen (HPF).

Of the various blood proteins measured, HPF was the only one which showed a linear relationship with antiheparin activity as assessed by multiple linear regression. It is suggested that increased antiheparin activity in rheumatoid arthritis might be due to the formation of a complex of heparin with HPF, thus reducing the amount of heparin available for interference with coagulation.

The possible clinical significance of these observations is discussed.

We are much indebted to Dr. J. Sharp for his help and encouragement in this work, and to Dr. R. V. Dent for his help in providing blood samples from healthy subjects in the older age groups. We are also grateful to Mr. H. Ellis for help in the statistical analysis of the results, and Miss A. Horne for technical assistance.

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L'activité antihéparinique et la fraction précipitable par l'héparine dans le plasma des malades atteints de polyarthrite rhumatoïde

RÉSUMÉ

L'étude de certaines altérations hématologiques chez 34 malades atteints de polyarthrite rhumatoïde démontra une augmentation significative de l'activité antihéparinique dans leur plasma, comparé à celui des témoins d'âge et de sexe similaires. On nota aussi une augmentation importante du taux de fibrinogène plasmatique et de la fraction du fibrinogène précipitable par l'héparine (*heparin precipitable fraction*=HPF).

D'entre toutes les protéines sanguines étudiées l'HPF seule accusa un rapport linéaire avec l'activité antihéparinique, évaluée par la régression linéaire multiple. On suggère que l'augmentation de l'activité antihéparinique dans la polyarthrite rhumatoïde peut être due à la formation d'un complexe héparine-HPF et à la réduction, de cette façon, de la quantité d'héparine disponible pour entraver la coagulation.

On discute la portée clinique de ces observations.

La actividad antiheparínica y la fracción precipitable por la heparina en el plasma de enfermos con poliartritis reumatoide

SUMARIO

Estudios de ciertas alteraciones hematológicas revelaron un aumento apreciable de la actividad antiheparínica en el plasma de 34 enfermos con poliartritis reumatoide en comparación con testigos de edad y sexo similares. Se notó también un aumento importante de las cifras del fibrinógeno plasmático y de la fracción del fibrinógeno precipitable por la heparina (*heparin precipitable fraction*=HPF).

De todas las proteínas sanguíneas medidas, sólo la HPF reveló una correlación lineal determinada por la regresión lineal múltiple. Se sugiere que el aumento de la actividad antiheparínica en la poliartritis reumatoide se puede deber a la formación de un complejo heparina-HPF y consiguiente reducción de la cantidad de heparina disponible para impedir la coagulación.

Se discute la posible importancia clínica de estas observaciones.