RESEARCH PAPERS

THE ANTICOAGULANT ACTIVITY OF DEXTRAN SULPHATE

II. THE EFFECT OF DEXTRAN SULPHATE ON THE ONE-STAGE PROTHROMBIN TIME

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IN a previous paper[1], the anticoagulant activity of dextran sulphate was compared with that of heparin. The experiments were designed to study the effects of the two drugs on various stages of the clotting sequence. This communication deals with the effects of the drugs on one simple and widely used clotting test, the one-stage prothrombin time[2,3].

As Biggs and Douglas[4] have pointed out, the so-called prothrombin time with brain thromboplastin does not necessarily measure prothrombin. In this test, the clotting time is prolonged by a reduction in clotting factors V, VII or, to a lesser degree prothrombin[4]; and also by a reduction in fibrinogen concentration lower than about 0·05–0·10 g. per cent[5]. There is evidence that the clotting time is shortened by an abnormal increase in factor V activity in certain cases of thrombosis[6] and by the increased titre of factor VII which occurs in pregnancy[7]. It is probable that the test proceeds in three stages: first, the brain reagent is activated by factor V and factor VII[8,9]; then, prothrombin is converted to thrombin; and finally, fibrinogen is converted to fibrin. Clearly, a lengthening or shortening of the prothrombin time can be caused by acceleration or delay in any one or more of these stages.

From the previous work[1] it may be inferred that, by comparison with heparin, dextran sulphate interferes but feebly with the reactions of the second and third stages of the prothrombin time test.

EXPERIMENTAL

Materials

Citrated plasma was obtained by centrifuging a mixture of 9 parts of normal venous blood and 1 part of 3·8 per cent. (w/v) trisodium citrate (anhyd.).

Human brain thromboplastin, acetone-dried extract[10].

Russell's Viper venom ("Stypven"), 1 : 10,000 reagent solution.

Dextran sulphate and heparin. As in the previous paper[1], in which details of the various preparations were given, the drug concentrations are expressed in terms of International Standard heparin (130 u./mg.) and British Standard dextran sulphate (on the basis of 25 heparin u./mg.).

Special reagents are described in connection with the experiments in which they were used.

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Methods

The performance of the prothrombin time test followed the usual principle[11], in which 0·1 ml. of citrated plasma received 0·1 ml. of brain or viper venom and 0·1 ml. of M/40 calcium chloride solution. It was sometimes convenient to prepare the brain or the viper venom in M/40 calcium chloride solution, so that the two reagents were added together in 0·1 ml. The final volume was usually restricted to the conventional 0·3 ml., although in some cases it was increased to 0·4 ml. but the general form of the test was not altered. The observations were made in a water bath at 37° C. As in the previous work, replicate readings, usually 4, were made in a balanced random order to eliminate systematic errors related to the passage of time[12].

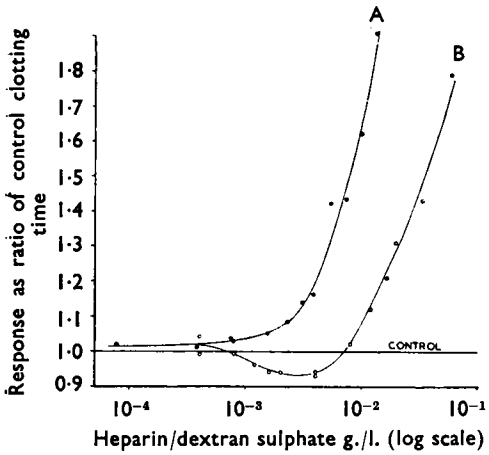

FIG. 1. *The accelerating effect of low concentrations of dextran sulphate.* (Experiment 1.) Each point represents the mean of 3–5 replicate readings of one-stage prothrombin time, expressed as a ratio of the mean control time obtained in the same experiment. Range of readings with 0·85 per cent. (w/v) sodium chloride solution ("saline control") in the six experiments represented, 19·9–22·6 sec. Commercial, clinical solution of heparin; Injection of Dextran Sulphate. A, heparin; B, dextran sulphate.

Where data are brought together from different experiments with differing control clotting times, the results are presented as the ratios of the test clotting times to the corresponding control clotting times. On the graphs, the control times are thus represented by horizontal lines at unity. The actual control clotting times (sec.) are then also given in the legends.

Experiment 1: *The accelerating effect of low concentrations of dextran sulphate.* Figure 1 shows the effect on the prothrombin time of varying concentrations of heparin or dextran sulphate; the other constituents of the test, and the final volume, were unaltered.

The heparin curve is much as might be expected, but the dextran sulphate graph shows that the drug causes a definite shortening of the clotting time at concentrations just less than those at which a lengthening is produced. This effect has varied in magnitude between 5 and 20 per cent. of the control time; and also, the range of concentrations over which it has been observed has varied from the 10-fold range of the Figure to about 100-fold, but with the shortest clotting times always occurring between 10^{-2} and 10^{-3} g./l. of dextran sulphate. Nevertheless, the effect has been clearly evident each time it has been sought.

The present paper is concerned with the investigation of this accelerating effect.

1. *Effect of varying the plasma and brain reagents*

i. *Experiment* 2: *Variation in brain reagent concentration.* Prothrombin times were obtained in the presence of 5 concentrations of dextran sulphate, using two concentrations of brain suspension, with the same concentration of plasma throughout. The results are illustrated in Figure 2, which shows that the acceleration was more marked with the higher concentration of brain.

ii. *Experiment* 3: *Variations in plasma concentration.* Prothrombin times were obtained with 5 concentrations of dextran sulphate using three concentrations of plasma and the same concentration of brain suspension throughout. The results (Fig. 3) showed that the degree of acceleration varied directly with the plasma concentration, over the tested range.

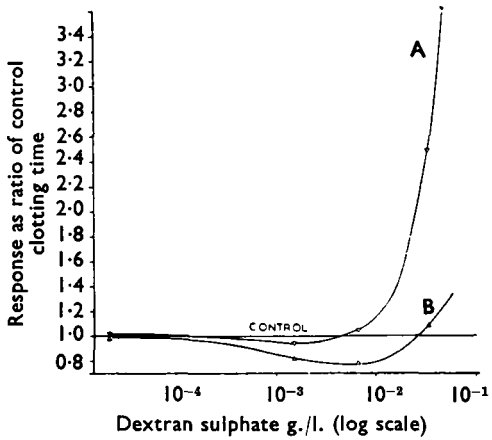

FIG. 2. *Variation in the concentration of the brain reagent.* (Experiment 2.) Each point represents the mean of 4 replicate readings of one-stage prothrombin time, expressed as a ratio of the mean control time with the same concentration of brain; the mean saline control readings were: with concentrated brain, A, 25·2 sec.; with brain diluted 1 : 50, B, 36·5 sec. British Standard dextran sulphate.

2. *Effect of the addition of heparin*

Experiment 4. Three concentrations of dextran sulphate were chosen so that the highest and lowest gave clotting times immaterially different from the control time, but in the presence of the intermediate concentration the clotting time was definitely shortened. Ten replicate readings were obtained of the control time alone, and of the clotting time with each of these concentrations of dextran sulphate both with and without the addition of a concentration of heparin sufficient to prolong the control clotting time to twice its original value. The mean results are given in Table I, and show that the proportion by which the clotting time is shortened by $1{\cdot}6 \times 10^{-3}$ g./l. of dextran sulphate is not varied by the anticoagulant effect of $6{\cdot}4 \times 10^{-3}$ g./l. of heparin. This suggests that the accelerating effect of dextran sulphate and the anticoagulant effect of heparin are not mutually exclusive.

3. *The Site of Action of the Accelerating Effect*

When the accelerating effect was noted in the prothrombin time test, attempts were made to detect an acceleration in the clotting time of whole blood. Tests were made both in glass tubes and silicone-coated tubes, but no suggestion of an acceleration was observed. Thrombin generation tests[13] were also made, with various concentrations of dextran sulphate less

TABLE I

COMBINED EFFECT OF ANTICOAGULANT CONCENTRATION OF HEPARIN WITH ACCELERATING CONCENTRATION OF DEXTRAN SULPHATE

Heparin concentration, g./l.	Dextran sulphate concentration, g./l.			
	0	$2{\cdot}0 \times 10^{-3}$	$1{\cdot}6 \times 10^{-3}$	$2{\cdot}3 \times 10^{-3}$
0	19·3 sec.	20·1 (+4 per cent.)	15·4 (−20 per cent.)	19·3 (±0 per cent.)
$6{\cdot}4 \times 10^{-3}$	39·8	39·6 (−1 per cent.)	32·7 (−18 per cent.)	41·0 (+3 per cent.)

Each entry is the mean prothrombin time in seconds derived from 10 replicate readings. The percentages in parentheses show the amounts by which the entries differed from the corresponding control clotting times obtained without dextran sulphate (Col. 2).
International Standard heparin: British Standard dextran sulphate.

than those used in anticoagulant tests reported in the previous paper[1], but using a similar method to eliminate bias related to order of testing, and again, acceleration was not observed. This was taken to mean that the affected reaction either did not occur in the spontaneous coagulation of blood or, that if it did, it occupied a very small proportion of the interval elapsing before thrombin appeared in the system.

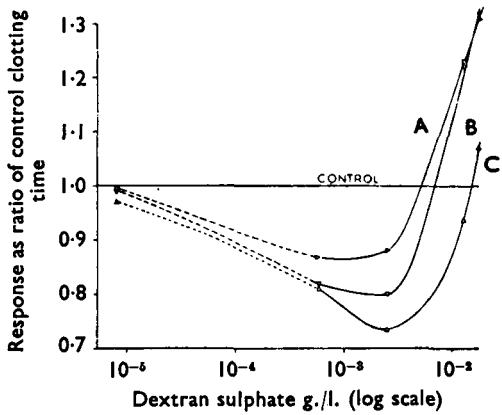

FIG. 3. *Variations in the concentration of plasma.* (Experiment 3.) Each point represents the mean of 3 replicate readings of one-stage prothrombin time, expressed as a ratio of the mean saline control time with the same concentration of plasma; the mean saline control readings were: 38 per cent. plasma C, 28·3 sec.; 19 per cent. plasma, B, 29·1 sec.; 9·5 per cent. plasma, A, 35·0 sec. In this experiment, 0·05 ml. of dextran sulphate solution was added to 0·15 ml. of plasma or diluted plasma; to this was added 0·1 ml. of brain suspension and 0·1 ml. of calcium chloride. The calcium chloride solutions were adjusted as follows: for 38 per cent. plasma, M/27; for 19 per cent. plasma, M/54; and for 9·5 per cent. plasma, M/108. British Standard dextran sulphate.

In the previous paper[1] evidence is given that dextran sulphate has no effect upon prothrombin conversion, and only a mild inhibitory effect upon the thrombin fibrinogen reaction. This suggests that in the prothrombin time test, the acceleration operates upon the reaction(s) between the brain reagent and factors V and VII[8,9]. To test this hypothesis the following experiments were made.

i. Experiment 5: *Experiment 1 repeated with various sources of "thromboplastin".* Prothrombin times were obtained with a single concentration of plasma, a range of concentrations of dextran sulphate, and with, in turn, brain extract, Russell's Viper venom and blood thromboplastin[14]. The results are given in Figure 4, which shows that the acceleration was equally well shown by brain and venom but was absent with blood thromboplastin.

ii. *Experiment* 6: "*Prothrombin time*" *with brain and purified clotting factors.* (*a*) A similar experiment was made by adding brain extract and calcium chloride to a mixture of purified clotting factors and a series of concentrations of dextran sulphate. The mixture contained fibrinogen (Lister Institute), the dialysed eluate from alumina[14] which had been incubated with normal plasma (as a source of prothrombin, the factor VII content being ignored[10]), the precipitate thrown down between 33 and 50 per cent. saturation of normal alumina-plasma with ammonium sulphate (as a source of factor V[10]) and normal serum (as a source of factor VII[10]), buffered with 0·0017M amino*tris*-(hydroxymethyl)methane[15] at pH 7·30. This confirmed (Fig. 5, *Direct* curve) that the acceleration occurred equally well in this mixture.

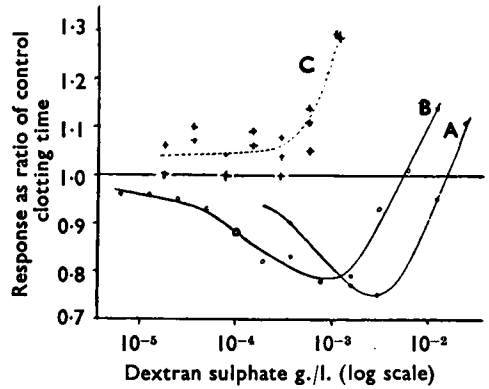

FIG. 4. *Different thromboplastic reagents.* (Experiment 5.) A: Brain suspension. Collected results from two series of tests: thus, each point represents mean of 2–6 readings of one-stage prothrombin time. Mean saline control clotting time, 25 sec.

B: Russell's Viper venom. Each point represents the mean of three replicate readings of one-stage prothrombin time. Mean saline control clotting time, 32 sec.

C: Blood Thromboplastin[14], prepared by incubating together alumina-plasma, serum, anti-hæmophilic globulin (as Lister Institute fibrinogen), washed platelets and calcium chloride solution. Each point represents a single reading, and the results are from three separate experiments with the same reagents. Mean saline control clotting time, 23 sec.

The results are shown as ratios of the corresponding saline control clotting times. With each thromboplastic reagent, 0·1 ml. was added to 0·1 ml. of citrated plasma and 0·1 ml. of dextran sulphate solution (prepared from a clinical Injection of dextran sulphate) or saline. Brain and venom were prepared in M/40 calcium chloride solution; in the tests with blood thromboplastin, a further 0·1 ml. of M/40 calcium chloride solution was added with the thromboplastin: the difference in final volumes has been allowed for in plotting the concentrations of dextran sulphate.

(*b*) The serum and factor V were then mixed with the brain and, after 3 min. incubation, sub-samples from this mixture were added to the remaining components. In five experiments, testing altogether a 1000-fold range of dextran sulphate concentrations, there was no evidence of an acceleration. Each experiment yielded duplicate readings against 5–8 drug concentrations, obtained in a balanced order to eliminate bias arising from instability of the activated brain. The mean results are shown in Figure 5, *Pre-incubated* curve.

iii. *Experiment* 7: *Effect of dextran sulphate on the rate of activation of brain suspension by factors V and VII.* Following Hardisty[9], brain suspension was incubated with diluted serum and a preparation containing factor V, and sub-samples were taken at intervals into mixtures of prothrombin and fibrinogen, and the clotting times recorded. Replicate

runs were made with and without dextran sulphate in the incubated mixture, at $1{\cdot}6 \times 10^{-3}$ g./l. This concentration lay at about the midpoint of the accelerating range in the experiment with purified factors (Experiment 6a, Fig. 5, *Direct* curve), and it was verified that it had a negligible effect on the clotting times of the prothrombin-fibrinogen mixtures, as in Experiment 6b, Figure 5, *Preincubated* curve. The results are shown in Figure 6, where an accelerating effect of the dextran sulphate is clearly seen. There was no effect upon the final activity of the incubated mixtures.

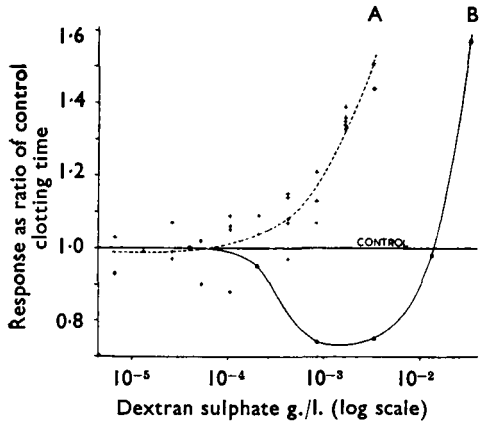

FIG. 5. *Tests with brain suspension and purified clotting factors.* (Experiment 6.) *Direct* curve (B): to 0·1 ml. dextran sulphate solution or saline were added 0·1 ml. quantities of solutions containing factor V, serum, prothrombin and fibrinogen respectively, and finally 0·1 ml. of a brain suspension in M/40 calcium chloride solution. Each point represents the mean of two readings. Mean saline control time, 18·4 sec.

Preincubated curve (A): Brain suspension was activated by incubation for 3 min. with factor V, serum and calcium chloride solutions. The mixture was then transferred to an ice bath and 0·1 ml. aliquots were tested against 0·2 ml. volumes of mixtures of prothrombin and fibrinogen with dextran sulphate solution or saline. Each point represents the mean of 2 readings; the data were derived from 5 batches of activated brain suspension. Mean saline control time, 14·5 sec. (Further points, obtained at lower drug concentrations but omitted from the graph, did not show a significant departure from the control clotting time.)

In each case the results are shown as ratios of the corresponding mean saline control clotting time for each run. Injection of Dextran Sulphate.

These experiments suggest that the prothrombin time is accelerated by a certain range of concentrations of dextran sulphate because under these circumstances the drug hastens the activation of the brain reagent by factors V and VII. Jenkins[16] found that Russell's Viper venom appeared to act as though it possessed the activities both of brain suspension and of factor VII. Figure 4 shows little difference between the acceleration produced by dextran sulphate when brain or venom were used, so that the acceleration would seem not to involve a reaction directly between factor VII and the thromboplastic reagent, but rather the reaction between brain and factor V, postulated by Hardisty[9], of which the rate is governed by factor VII[9].

4. *The role of* N-*sulphate and* O-*sulphate in the Accelerating Effect*

There is evidence that in heparin the sulphate groups are attached both by *N*-[17,18] and by *O*-linkages[19], whereas in dextran sulphate, the attachment is, of course, by *O*-linkages only[20]. It is believed that the *N*-sulphate linkages in heparin may be selectively broken by gentle acid hydrolysis[21],

which yields a derivative of low anticoagulant activity[17] known as ψ-heparin[22].

A series of ψ-derivatives were obtained from heparin after successive periods of acid hydrolysis (1 per cent. w/v solution of heparin in 0·04N HCl at 100° C.). Samples were withdrawn at 8-minute intervals up to

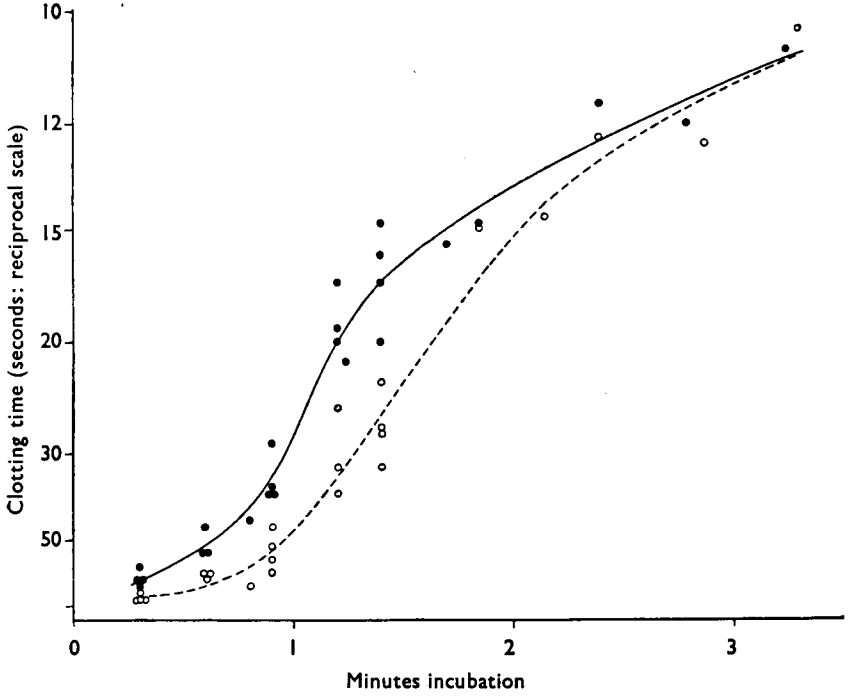

FIG. 6. *The effect of dextran sulphate upon the rate of activation of brain suspension by factors V and VII.* (Experiment 7.) Brain suspension was incubated with serum, a preparation of factor V, calcium chloride solution, and amino-*tris*(hydroxymethyl)-methane buffer, pH 7·3, 0·00625M[15]. The reaction was sampled at intervals into aliquots of a mixture of prothrombin and fibrinogen (following Hardisty[9]), of which the clotting times were recorded, and are shown reciprocally on the ordinate.

Unbroken Line: the incubated reaction contained dextran sulphate (prepared from the Injection), $1{\cdot}6 \times 10^{-3}$ g./l., added in the buffer.

Broken Line: the incubated reaction contained the buffer only.

The data represent 10 runs, 5 with and 5 without dextran sulphate. The drug concentration tested was chosen to lie at about the mid-point of the acceleration of the *Direct* (B) curve of Figure 5.

48 minutes; the 40-minute sample is not reported because its activity differed little from that of the 48-minute sample. The derivatives were isolated by freeze-drying after neutralisation and dialysis. Half the *N*-sulphate groups had been hydrolysed in *ca.* 20 minutes and all in 90 minutes, so that over the tested range, the derivatives would have retained 40–90 per cent. of the original *N*-sulphate groups (Dr. A. B. Foster, personal communication). Dose-response curves were obtained in the thrombin-plasma clotting time[23] and prothrombin time tests.

Experiment 8: *Dose-response curves in the thrombin-plasma clotting time test.* The 32 and 48 minute samples showed no anticoagulant activity, but dose-response curves obtained in the thrombin-plasma test with the earlier samples and with dextran sulphate are shown in Figure 7, where the ψ-series exhibits a progressive fall in the slope of the curves, passing from the high slope given by the parent heparin towards the low slope characteristic of dextran sulphate[1]. There is also apparent a progressive fall in anticoagulant activity shown by the positions of the curves.

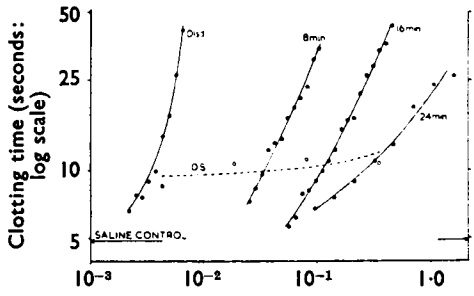

FIG. 7. *Thrombin-plasma clotting times as dose-response curves with dextran sulphate, heparin and hydrolysed heparins (ψ-series).* (Experiment 8.) The concentrations of plasma and thrombin were constant, and gave a saline control clotting time of 4·9 sec.; 0·2 ml. plasma received 0·1 ml. drug solution or saline, followed by 0·1 ml. human thrombin (Lister Institute) dissolved in M/20 calcium chloride solution. Each point represents the mean of 4 replicate readings.

DS: dextran sulphate, British Standard.

Dlsd: the parent heparin, dialysed only.

8 min., 16 min., 24 min.: ψ-samples obtained after these periods of hydrolysis.

Experiment 9: *Dose-response curves in the prothrombin time test.* Similar dose-response curves obtained in the prothrombin time test are shown in Figures 8 and 9. Figure 8 shows the results of a single experiment which determined the positions of the curves given by the various ψ-samples: the findings are similar to those of the thrombin-plasma test. In Figure 9 are shown, separately for each of the last 4 ψ-samples tested, the collected results of a number of experiments in which the accelerating effect was particularly studied. It may be seen that as the anticoagulant potency falls, the acceleration appears and increases progressively along the series, until finally the acceleration alone remains.

As a corollary to these experiments, tests were made with polyvinyl amine sulphate, which is a long-chain molecule carrying *N*-sulphates only; and, for comparison, also with polyvinyl alcohol sulphate, carrying *O*-sulphates only. An acceleration of prothrombin time was not observed with either drug.

DISCUSSION

These experiments have investigated the acceleration of the prothrombin time by a certain range of concentrations of dextran sulphate. The results suggest that this acceleration is effected by hastening the preliminary reaction(s) occurring between the brain reagent and factors V and VII, and that the phenomenon is a function of *O*-sulphate. A comparable phenomenon could not be detected when blood clotted spontaneously, and the acceleration of the prothrombin time test is not therefore thought to be of clinical significance.

Two theoretical possibilities now arise:—

1. The accelerating and the anticoagulant activities of dextran sulphate may depend upon different mechanisms, exerted independently upon the clotting system. Over the anticoagulant range of concentrations both effects might be present together but the acceleration would now be masked by the anticoagulant activity. The observed dose-response curve would thus be the resultant of the two opposing effects, but if the anticoagulant effect could be plotted alone, this curve would be expected to lie further down the drug concentration axis. An anticoagulant curve was in fact observed in this position in the experiments with blood thromboplastin (Experiment 5: Fig. 4) and activated brain suspension (Experiment 6b: Fig. 5, *Pre-incubated* curve) in both of which the reactions involving factors V and VII had occurred before dextran sulphate was added to the system. Similarly, the acceleration plotted alone would be expected to be even greater than that observed in the present experiments, but no method of doing this was devised. This hypothesis is illustrated in the upper part of Figure 10.

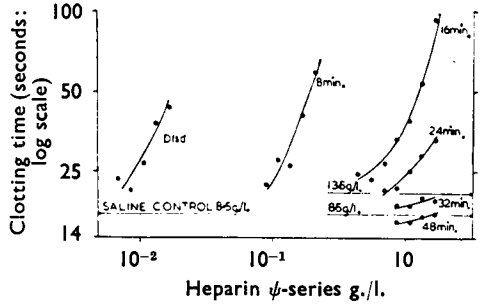

FIG. 8. *One-stage prothrombin times as dose-response curves with heparin and hydrolysed heparins (ψ)-series.* (Experiment 9.) The concentrations of plasma and brain reagent were constant and gave a control clotting time with 0·85 per cent. saline of 17·3 sec.; 0·1 ml. plasma received 0·1 ml. drug solution or saline, followed by 0·1 ml. brain suspension in M/40 calcium chloride solution. Each point represents the mean of 2 replicate readings.

When testing the higher drug concentrations (which were all added to the reaction in saline) it was thought that the total solute concentration might have been high enough to have prolonged the clotting time. A second series of control readings was therefore made with 1·35 per cent. (w/v) sodium chloride solution (to represent 0·85 per cent. (w/v) sodium chloride solution + 0·50 per cent. drug concentration, which was the highest prepared: this yielded the highest concentration on the graph, 0·16 per cent., when diluted with the plasma and brain suspension), and the mean of these readings was 21·1 sec. If this control value is used for comparison with the clotting times obtained with the highest drug concentration plotted, the acceleration produced by the 48 min. sample is still more striking.

Dlsd: the parent heparin, dialysed only.

8 min., etc.: ψ-samples obtained after these periods of hydrolysis.

If this were true, the findings in Experiments 2 and 3 (Figs. 2 and 3) might be explained by supposing that variations in the concentrations of plasma or brain suspension altered the relative position of the accelerating and anticoagulant curves, and so affected the shape of the observed resultant. The data of Experiment 4 (Table I) are clearly also in line with this hypothesis, because they show that the accelerating effect of dextran sulphate can still be observed in the presence of an anticoagulant concentration of heparin.

2. On the assumption that a progressive reduction in *N*-sulphate is

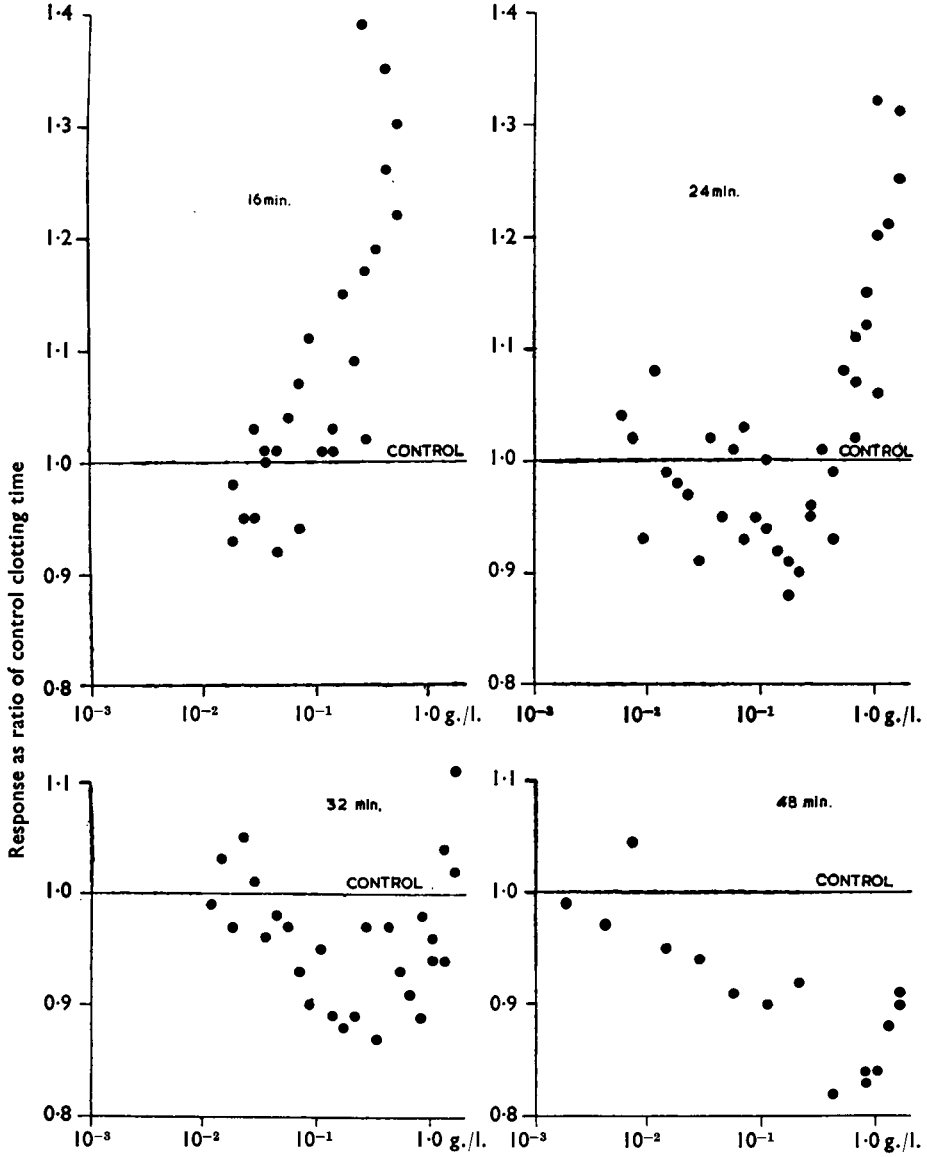

FIG. 9. *The acceleration of the prothrombin time by hydrolysed heparins (the last 4 samples in the ψ-series).* (Experiment 9.) The conditions were as for Figure 8. Each point represents a single reading, and the data are collected from 7 experiments in which the saline control clotting times lay between 18·2 sec. and 21·8 sec. In each case the drugs were added in saline, and the control readings were obtained with a concentration of saline corresponding to the total solute concentration in g./l. 16 min., etc.: ψ-samples obtained after these periods of hydrolysis.

the only change induced in heparin by gentle acid hydrolysis, the experiments with the ψ-derivatives suggest that heparin may possess an homologous biphasic activity, by virtue of its *O*-sulphates. (In fact, some *O*-sulphate linkages may also have been broken (Dr. A. B. Foster, personal communication), which might have the effect of reducing still further the anticoagulant activity of the resulting compound.) Comparison of Figures 1 and 9 shows that the accelerating effect of the ψ-derivatives occurs at a much higher drug concentration (*ca.* 500-fold) than the accelerating effect of dextran sulphate. A comparison between the dose-response curves obtained with untreated heparin (Fig. 1), and with dextran sulphate in the "preincubated" experiments (Figs. 4 and 5), suggests that the purely anticoagulant activities of the two drugs would be of the same order of potency in the prothrombin time test. This hypothesis is illustrated in the lower part of Figure 10, which shows the observed resultant curves for untreated heparin, the dialysed parent sample of the ψ-series and the ψ-derivatives. Clearly, the accelerating effect of untreated heparin would be masked by the anticoagulant effect. It has been suggested that the anticoagulant activity of untreated heparin largely depends upon the *N*-sulphates[20,24]. On this hypothesis, the anticoagulant activity of the *O*-sulphates of heparin might only be observed with higher drug concentrations that have been tested. This would explain why the 48 minutes ψ-sample appears to have no anticoagulant activity.

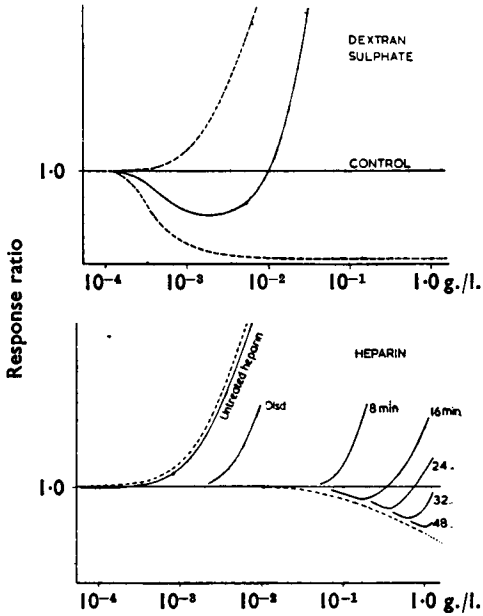

FIG. 10. *Curves illustrating the hypothesis of independent accelerating and anticoagulant activities of dextran sulphate and heparin in the one-stage prothrombin time test.* The full line represent the observed dose-response curves, plotted as ratios of the control clotting times. The broken lines represent the hypothetical anticoagulant and accelerating activities respectively, plotted in the same way. For the dextran sulphate curves, *cf.* Figures 1, 4 and 5. For the heparin curves (which include the observed curves with the ψ-series), *cf.* Figures 1, 8 and 9: data suggesting the dotted portion of the broken curve in the heparin figure were obtained from an independent ψ-sample which accelerated the prothrombin time by about 25 per cent. at the highest tested concentration of 1·6 g./l.

SUMMARY

1. At drug concentrations immediately below the anticoagulant range, the one-stage prothrombin time was accelerated by dextran sulphate to

about 10 to 20 per cent. The magnitude of the effect varied between different batches of reagents, and with variations in the concentrations of plasma and brain suspension. The acceleration could still be detected in the presence of heparin in anticoagulant concentration, and was also seen with Russell's Viper venom.

2. The acceleration was absent, and the anticoagulant potency of dextran sulphate was increased, if the test was made with blood thromboplastin, or with brain suspension activated by preincubation with clotting factors V and VII. Dextran sulphate was found to increase the rate of activation of brain suspension by these factors, and this was thought to explain the acceleration of the ordinary test.

3. The acceleration was not detected in the spontaneous coagulation of blood, and is not therefore thought to be of clinical significance.

4. A similar acceleration was observed with ψ-derivatives of heparin obtained by acid hydrolysis, a process which is believed selectively to break *N*-sulphate linkages but not to greatly affect *O*-sulphate linkages.

5. It is suggested that with both dextran sulphate and heparin there are separate accelerating and anticoagulant effects, and that the observed dose-response curves are the resultants of these opposing activities. On this view the accelerating effect of dextran sulphate operates at slightly lower concentrations than the anticoagulant effect, producing the observed biphasic curve; with heparin, on the other hand, the anticoagulant effect (of *N*-sulphate) is exerted at far lower concentrations than the accelerating effect (of *O*-sulphate), and so with untreated heparin the acceleration is completely masked.

We are indebted to Dr. C. R. Ricketts for his advice on the chemical aspects of this investigation, and for having provided samples of polyvinyl alcohol and amine sulphates; to Prof. M. Stacey and to Dr. A. B. Foster for having provided the series of ψ-heparin samples and the details of their preparation; and to Dr. R. G. Macfarlane for having suggested Experiments 2, 3 and 4.

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