THE ANTICOAGULANT ACTIVITY OF DEXTRAN SULPHATE

I. AN *in vitro* COMPARISON BETWEEN THE ACTIONS OF DEXTRAN SULPHATE AND HEPARIN ON THE VARIOUS STAGES OF BLOOD COAGULATION

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THE anticoagulant properties of synthetic polysaccharide sulphuric acid esters were first reported by Bergström[1,2]. The sodium salt of dextran sulphate was studied by Ricketts and Walton[3], who found that dextran sulphates of low molecular weight were no more toxic than heparin, and that the greatest anticoagulant effect was attained when the number of sulphate groups exceeded 1·3 per glucose unit. Ricketts[4] also found that a chain length of 5 to 20 glucose units was most suitable for therapeutic use.

A clinical trial with two samples of dextran sulphate having these characteristics has been reported by Ricketts, Walton, van Leuven, Birkbeck, Brown, Kennedy and Burt[5]. The dosage was based on that of heparin, the anticoagulant activity of the dextran sulphate having been assayed against heparin by a method employing the clotting time of recalcified, citrated sheep plasma[6]. This assumed, of course, that the anticoagulant actions of dextran sulphate and heparin were fundamentally similar, but a comparison of results from various centres[7] revealed interesting differences between the relative anticoagulant potencies of the two drugs when assayed by various methods. A comparative study of the anticoagulant properties of heparin and dextran sulphate was therefore undertaken.

EXPERIMENTAL

Materials

Dextran Sulphate. An Injection of dextran sulphate prepared for the clinical trial[5] (manufacturer's number, 52DSO39) and the British Standard dextran sulphate (Author's Preparation I_4[4]) were both tested. The preparations behaved similarly; the Injection was assayed against the British Standard by the one-stage prothrombin time test and subsequent results are expressed in absolute concentrations of the latter; in each experiment an indication is given of which preparation was used.

Based on overall anticoagulant activity, numerous assays against International Standard heparin by various methods in 11 laboratories yielded a mean estimate of heparin potency for the British Standard of 25 u./mg.[7] which is about one-fifth of the potency of International Standard heparin (130 u./mg.[8]). Thus, in the following charts, a comparison between the two drugs on the basis of overall anticoagulant activity (clinical unitage) may be obtained by imagining the position of

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the dextran sulphate curves to be moved to the left on the drug concentration axis through a five-fold interval.

Heparin. The majority of the experiments were made with International Standard heparin, although in some, another powder heparin or a commercial, clinical solution were used. The three samples behaved similarly in the tests in which they were compared, and the results are presented as absolute concentrations of the International Standard, by using conversion factors obtained from an assay of the "powder" (one-stage prothrombin time test) and from the labelled potency of the commercial preparation. In each case it is stated which sample was used.

Thrombin and Fibrinogen. Lyophyllised human thrombin and fibrinogen were obtained from the Lister Institute and dissolved in 0·85 per cent. (w/v) sodium chloride solution ("saline") as directed on the ampoules. The potencies of the thrombin solutions were calculated from the labelled unitages. Veronal buffer[9] was added to the fibrinogen solutions because this was found to prevent precipitation when the solutions were repeatedly frozen and thawed.

Blood and Plasma. Human blood was obtained from healthy adult subjects or, in a few cases, from hospital patients not acutely ill. Nine volumes of blood were mixed with one volume of 3·8 per cent. (w/v) trisodium citrate (anhyd.) to obtain *citrated whole blood*: *citrated plasma* was obtained from this by centrifugation sufficient, unless otherwise stated, to remove the majority of the platelets.

Methods

Experiments were designed to compare the anticoagulant activities of heparin and dextran sulphate on various phases of the coagulation mechanism, and techniques were selected for testing limited portions of the clotting sequence. In presenting the results, it is convenient to consider the processes of clotting in the reverse order to that in which they naturally occur, because this allows the simpler tests to be considered first.

In the one-stage tests, replicate readings (usually 2 or 4) were made in a symmetrical sequence to eliminate bias due to progressive changes in any of the reagents[10]. With the two-stage tests, this plan could not always be followed but the control runs were repeated towards the end of those experiments in which changes in reagents might have produced the observed effects in test runs, and the important runs were repeated on another occasion before the results were accepted.

The reagents were kept in an ice bath until immediately before each test, and, with one exception to be noted, the clotting times were measured in a water bath at *ca.* 37° C. The anticoagulants, usually in saline, or occasionally in calcium chloride solution (when this was required in the system), were added to the reactions immediately beforehand.

Part 1: *The Reaction between Thrombin and Fibrinogen*

(i) *The thrombin clotting time of citrated plasma*[11]

Figure 1 shows the responses obtained with thrombin over a range of concentrations of the two drugs in citrated plasma. The observable

range of responses was passed through in rather less than a ten-fold range of heparin concentrations, but the same change in response occupied approximately a ten-thousand-fold range of dextran sulphate concentrations, only a part of which is shown. (Figure 1 suggests that at lower concentrations the response curve would cross, but in another experiment which tested the minimal interfering concentrations of both drugs, this was not observed: the dextran sulphate curve reached the control clotting time at an absolute concentration of about five times that required for the minimal effect of heparin.) The difference between the slopes of the response curves was somewhat diminished if the thrombin concentration was reduced but was clearly observable throughout the useful working range.

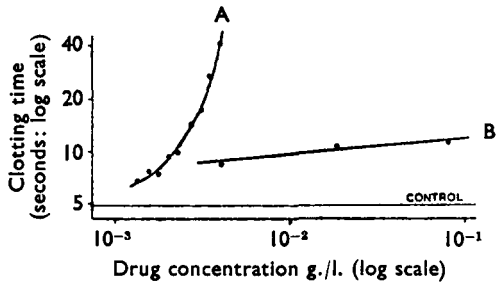

FIG. 1. *Part* 1: *The reaction between thrombin and fibrinogen.* The thrombin clotting time of citrated plasma. Final concentration of thrombin *ca.* 12 u./ml.; mean saline control clotting time, 4·9 sec. "Powder" heparin: British Standard dextran sulphate.
A. heparin; B. dextran sulphate. Each point is the mean of 4 readings.

(ii) *Interaction with a co-factor*

In a number of experiments, increasing concentrations of plasma (or serum) were added to clotting systems containing fixed concentrations of purified thrombin and fibrinogen and various concentrations of dextran sulphate or heparin.

Three differences between heparin and dextran sulphate were found in these experiments. First, the drugs differed in the minimal concentration of plasma required to prolong the clotting time of the purified system; with dextran sulphate, the clotting time did not lengthen until the concentration of plasma was raised to two to four times that required by a concentration of heparin giving a comparable clotting time in concentrated plasma. Occasionally, the higher plasma concentrations produced a negligible increase of clotting time with dextran sulphate. Second, in the effective (upper) range of plasma concentrations, to obtain a given increase in anticoagulant effect, a far greater increase (in Figure 2, approximately 12-fold) in concentration was required with dextran sulphate than with heparin: this clearly reflects the finding of the previous experiment. These two effects are shown in the data plotted in Figure 2.

In addition, dextran sulphate inconstantly lengthened the clotting times obtained with the lower plasma concentrations (peak at about 1 per cent. of plasma). This effect was not seen with heparin.

Additive Effect of Dextran Sulphate and Heparin. Figure 3 shows the results of testing both separately and together similar absolute concentrations of the two drugs. While this concentration of dextran sulphate

alone appeared not to interact with the co-factor, it clearly potentiated the interaction between heparin and the co-factor.

These experiments suggested that dextran sulphate reacted weakly with a co-factor similar to that required for heparin and did not block the reaction between heparin and its co-factor.

(iii) *Interaction with progressive antithrombin*

(*cf.* Antithrombin III in the classification of Seegers[12].)

Citrated plasma was defibrinated by the addition of a small quantity of thrombin. Observations were then made of the rate of decay of a larger quantity of thrombin, subsequently added[13] in the presence of various concentrations of heparin or dextran sulphate. The lowest concentration of heparin causing a definite increase in the decay rate appeared to be about 4×10^{-4} g./l., whereas with dextran sulphate about 8×10^{-3} g./l. appeared to be required to give a like effect. (These tests were made at 15° C. to obtain a slower reaction.)

These results suggest that heparin delays the clotting of plasma by thrombin partly by interfering directly with the thrombin-fibrinogen reaction, and partly by accelerating the destruction of thrombin by progressive antithrombin. The actions of dextran sulphate appear to be similar but very much weaker.

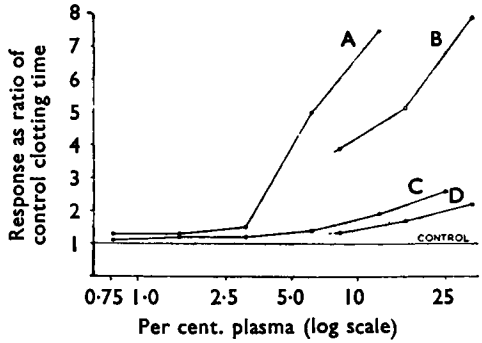

FIG. 2. *Part* 1: *Interaction with a co-factor.* The clotting times of mixtures of purified thrombin and fibrinogen with varying concentrations of plasma, and of heparin or dextran sulphate. For each drug, the thrombin concentration was constant. Control clotting times: for heparin, 6·6–8·7 sec.; for dextran sulphate, 6·3–6·8 sec. (there was some variation in control clotting times at different plasma concentrations, and the response ratios were calculated from the control clotting times obtained at the corresponding plasma concentrations). International Standard heparin: Injection of dextran sulphate. Each point is the mean of 2 readings.

A. Heparin $3{\cdot}0 \times 10^{-3}$ g./l.
B. Dextran sulphate 2·2 g./l.
C. Heparin $1{\cdot}5 \times 10^{-3}$ g./l.
D. Dextran sulphate $9{\cdot}0 \times 10^{-2}$ g./l.

Part 2: *The Conversion of Prothrombin to Thrombin*

(i) *In spontaneous coagulation*

Using the Thrombin Generation Test of Macfarlane and Biggs[14], curves were obtained showing the liberation and decay of thrombin after the recalcification of citrated, whole blood, with and without various concentrations of the anticoagulants. Blood samples from 22 subjects were each tested with both drugs and the results obtained are shown in Figures 4 and 5, with the controls obtained on the same samples. The drug curves have been corrected for the effects of the anticoagulants upon the fibrinogen used as indicator of thrombin concentration, by repeating the

tests on further portions of the same samples with corresponding concentrations of the drugs in the fibrinogen instead of in the clotting blood. For each drug concentration, the mean difference between the clotting times of the control curve (no drug) and of the curve obtained with the drug in the indicator, was then added to each reading obtained in the corresponding drug curve. For each sample of citrated blood the five runs were obtained in a random order. Each citrated sample was kept in a chilled, silicone-coated container until required for each run (longest storage, about 4 hours).

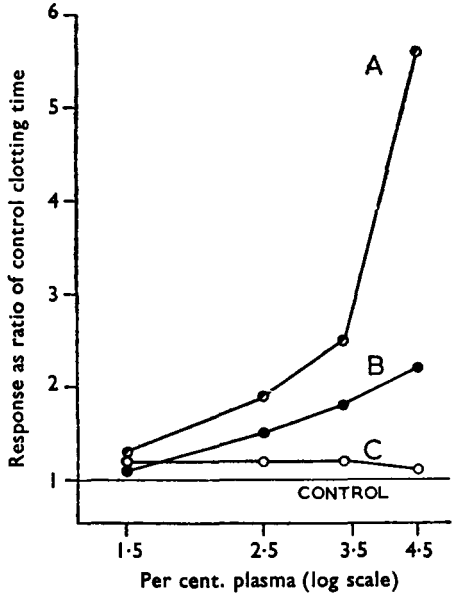

FIG. 3. *Part* 1: *Interaction with a co-factor.* The clotting times of mixtures similar to those of Figure 2, with the same thrombin concentration throughout. Control clotting times 14·7–15·4 sec. International Standard heparin; British Standard dextran sulphate. Each point is the mean of 4 readings.

A. Heparin + dextran sulphate
B. Heparin $4{\cdot}6 \times 10^{-4}$ g./l.
C. Dextran sulphate $5{\cdot}7 \times 10^{-4}$ g./l.

The series of curves with heparin was observed up to the point of extinction because a concentration of the drug sufficient to delay the liberation of thrombin beyond about 30 minutes proved also sufficient to depress the quantity liberated below the limit of observation. With dextran sulphate, on the other hand, a delay as great as about 45 minutes was readily observed, because even under these conditions there was practically no diminution in the quantity of thrombin formed. Indeed, the series only stops at this point because the stock batch of fibrinogen was then exhausted and subsequent samples differed in their reaction to thrombin and would therefore not have given comparable results. In any event it is clear that the quantity of thrombin appearing in the system is far less affected by dextran sulphate than by heparin.

(ii) *In the two-stage prothrombin time test*

Figure 6 shows the results of a two-stage prothrombin time test with the highest concentrations of heparin and dextran sulphate which gave workable clotting times in the thrombin indicator. In each case, these concentrations were sufficient definitely to prolong the one-stage prothrombin time, although the heparin effect was the greater. In calibrating the indicator with known thrombin concentrations, allowance was made for the inhibitory effects of the anticoagulants.

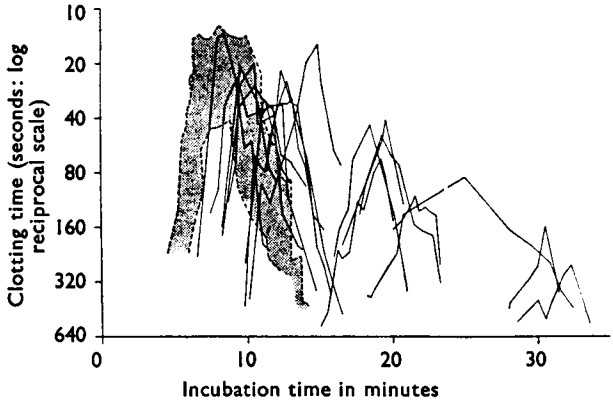

FIG. 4. *Part* 2: *Thrombin generation tests with various concentrations of heparin* (the concentrations are given in Figure 8): citrated whole blood. Time-scale from recalcification. The reciprocal clotting time-scale is proportional to thrombin concentration: this is plotted logarithmically to simplify the shape of the curves. The control curves obtained from the same blood samples fell within the hatched area. International Standard heparin. Each curve shows a single series of readings.

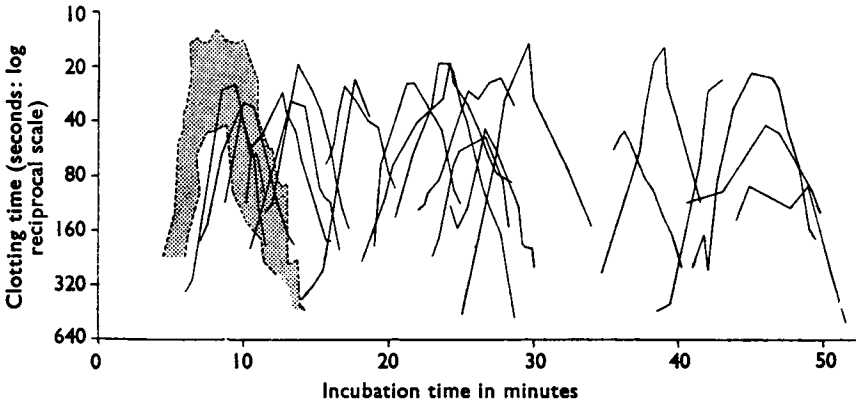

FIG. 5. *Part* 2: *Thrombin generation tests with various concentrations of dextran sulphate* (the concentrations are given in Figure 8). Plotted as Figure 4. Injection of dextran sulphate.

With dextran sulphate, the point of extinction was reached in the indicator reaction at lower concentrations than in the main reaction, but interference with thrombin liberation was not observed up to this point. As Figure 6 shows, the results with heparin followed closely the family of curves published by Biggs and Douglas[15]. In further tests with a different batch of brain extract, the quantity of thrombin liberated passed from the control level to extinction within a heparin range of approximately 8×10^{-5} to 8×10^{-4} g./l.; with dextran sulphate, a delay in the appearance of thrombin was observed in an experiment testing $3{\cdot}2 \times 10^{-3}$ g./l., but even in this case there was little reduction in the quantity of thrombin which appeared.

These results were thus very similar to those obtained with the thrombin generation test, and supported the finding that dextran sulphate did not interfere with prothrombin conversion over the tested range.

Part 3: *The Development of Blood Thromboplastin*

Biggs, Douglas and Macfarlane[16] showed that a powerful thromboplastin developed in clotting blood, and that it could be prepared from a reaction mixture composed of platelets and serum together with plasma which had been freed of prothrombin by treatment with alumina. The active constituents of this mixture were shown to be antihæmophilic globulin and factor V (supplied by the treated plasma), Christmas factor and factor VII (present in the serum), platelet factor(s), and calcium. The effect of heparin upon the development of blood thromboplastin was also studied, and guided by this work[17], the effects of heparin and dextran sulphate were compared in the following experiments.

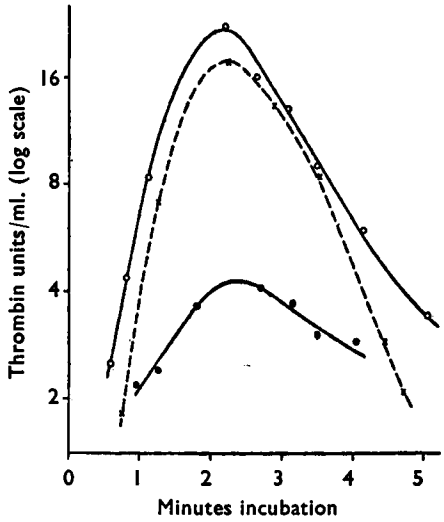

FIG. 6. *Part* 2: "*Two-stage*" *prothrombin time test*, with highest workable concentrations of heparin (2×10^{-3} g./l.: clinical solution, solid circles) and dextran sulphate (8×10^{-3} g./l.: Injection, open circles) compared with saline control (broken line).

(i) *Relative overall effects of the two drugs*

(*a*) *In spontaneous coagulation.* Accepting the proposition of its originators[14] that the thrombin generation test may broadly be interpreted in terms of the generation of blood thromboplastin, information on the early stages of coagulation may be obtained from Figures 4 and 5. The data for both drugs have been summarised in two ways in Figures 7 and 8.

In Figure 7, the shortest observed clotting time (corrected, as explained above) in each of the thrombin generation tests have been plotted against the times at which they occurred. This presentation therefore shows in one diagram the main information to be obtained from Figures 4 and 5, namely that heparin reduces the quantity of thrombin liberated in proportion as the appearance of thrombin is delayed, whereas dextran sulphate merely delays the appearance of thrombin over the range of drug concentrations that were tested.

In Figure 8, the times of observing the shortest clotting times are plotted against the corresponding drug concentrations. The points from the two drugs appear to fall on broadly parallel regressions separated by an approximately four-fold dose interval. This agrees well with the

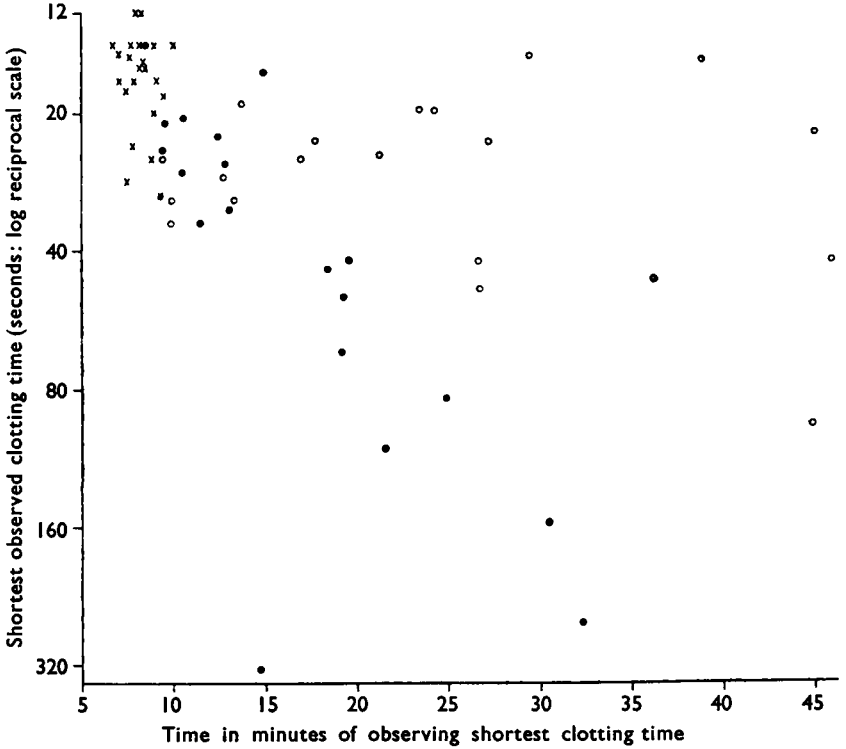

FIG. 7. *Part* 3: *Development of blood thromboplastin.* The data of Figures 4 and 5 replotted as indicated on the co-ordinates. Solid circles represent the heparin curves, open circles the dextran sulphate curves, and crosses the saline control curves.

mean potency ratio of approximately five-fold derived from the assays previously mentioned[7]. Figures 1, 7 and 8, taken together, strongly suggest that the anticoagulant effect of dextran sulphate on spontaneous coagulation chiefly depends on interfering with the development of blood thromboplastin.

(*b*) *In the generation of blood thromboplastin from active constituents.* Figure 9 shows the results of an experiment in which thromboplastin generation tests were carried out in the presence of various concentrations of the two drugs. The measurement of thromboplastin production is derived from the area under the graph by which the results of this test are ordinarily studied: this method of summarising the results gives expression to variations both in the rate of thromboplastin generation and in the final titre of activity which is developed[18]. As in the other two-stage tests, the effects of the anticoagulants were allowed for when the calibration curves for these experiments were prepared.

The figure shows a linear relationship between thromboplastin production and the logarithmic concentrations of both drugs. The difference between the positions of the curves approximately corresponds to the five-fold difference in overall anticoagulant activity previously mentioned,

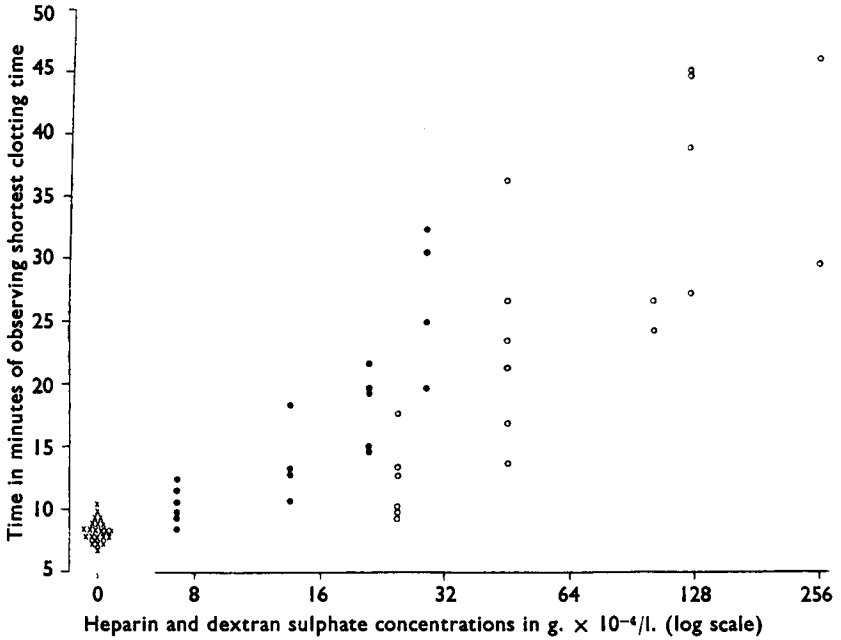

FIG. 8. *Part* 3: *Development of blood thromboplastin.* The data of Figures 4 and 5 replotted as indicated on the co-ordinates. Solid circles represent the heparin curves, open circles the dextran sulphate curves, and crosses the saline control curves.

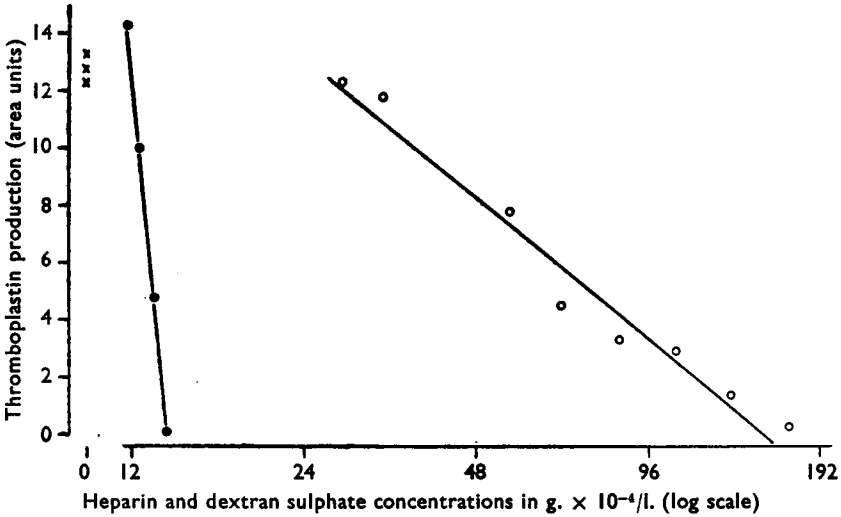

FIG. 9. *Part* 3: *Thromboplastin Generation Tests*, summarised as described in the text. Alumina-treated plasma[16], 4 per cent.; serum (not previously diluted), 2·5 per cent., buffered with amino*tris*(hydroxymethyl)methane 0·02 M, pH 7·3[25]. Heparin, International Standard, solid circles; dextran sulphate, British Standard, open circles; three control curves are represented by crosses.

but this adjustment does not, of course, eliminate the difference between the slopes of the curves, which contrasts strongly with the similarity between the corresponding slopes of Figure 8. However, reference to Figures 4 and 5 shows that with one exception there is little widening of the thrombin curves as either series passes to the right, which suggests that over the tested range there was no demonstrable alteration in the activity of the thromboplastin which was formed, but only a delay in its generation (*cf.* Figures 5c and 8 of Biggs and Douglas[15]; and Figure 1B of Ingram[19]). Thus, from Figure 8 it may be inferred that in blood clotting spontaneously, these drugs have a similar activity in delaying the generation of blood thromboplastin (at any rate in the low concentrations tested); but Figure 9 suggests that the quantity of thromboplastin formed is less affected by dextran sulphate than by heparin.

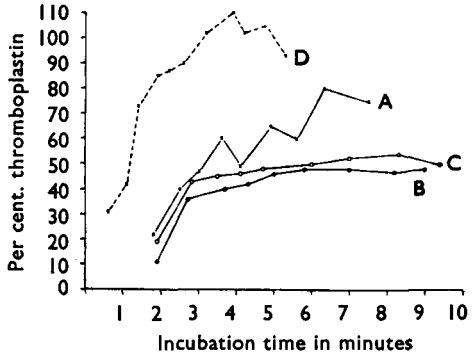

FIG. 10. *Part* 3: *Thromboplastin generation tests*, with platelets, alumina-treated plasma[16], serum and calcium chloride solution, buffered with amino*tris*(hydroxymethyl)methane 0·02 M, pH 7·3[25].

A. control curve
B. with $1{\cdot}06 \times 10^{-3}$ g./l. International Standard heparin.
C. with $1{\cdot}80 \times 10^{-3}$ g./l. British Standard dextran sulphate.
D. alumina-plasma, serum and calcium chloride preincubated for 5 min. before adding the platelets (at 0 min.).

(ii) *Relative effects on Reactions not involving Platelets*

When studying preliminary reactions leading to the generation of thromboplastin, Biggs, Douglas and Macfarlane[20] developed the technique of preincubating calcified mixtures of some of the reagents to which the remainder were added after an interval. The curve of the thromboplastin that was then generated was compared with the curve obtained when all the reagents were mixed at the same time, and it was found that preincubation of certain mixtures lead to a more rapid liberation of thromboplastin when the remaining reagents were added. In these cases it was supposed that preliminary, time-consuming reactions had taken place between the components which were preincubated together, so that when the thromboplastin system was completed, the usual latent interval was reduced. This technique was used in some of the following experiments.

In Figure 10 is shown the curve of thromboplastin generation obtained in the ordinary way from a mixture of alumina-treated plasma[16] (4 per cent.), platelets, serum (2·5 per cent., not previously diluted) and calcium chloride solution (the control curve). Concentrations of the two drugs were chosen which produced a similar and relatively small inhibition: these curves fall together somewhat below the control curve. The Figure

also includes the curve obtained on adding the platelets to a mixture of the other components which had been preincubated (without drugs) for 5 minutes. These four curves are each to be regarded as controls of different sorts for those of the following figure. Figure 11 shows four curves which were all obtained in the same way as the preincubation curve of Figure 10 except that the chosen concentration of each drug was added either at the beginning of the preincubation or with the platelets.

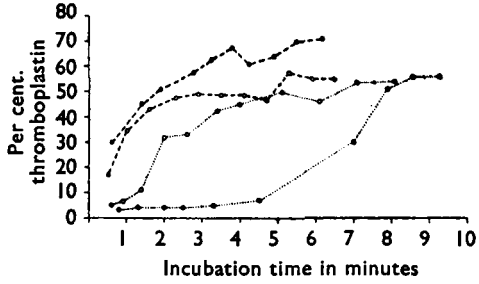

FIG. 11. *Part* 3: *Thromboplastin generation tests*, with alumina-plasma, serum and calcium chloride preincubated for 5 min. before adding platelets, and buffered with amino*tris*(hydroxymethyl)methane, 0·022 M, pH 7·3[25].

●·····● Heparin, $1{\cdot}16 \times 10^{-3}$ g./l. in the preincubated mixture.

○·····○ Dextran sulphate, $1{\cdot}97 \times 10^{-3}$ g./l. in the preincubated mixture.

The addition of platelets reduced these drug concentrations to 1·06 and $1{\cdot}80 \times 10^{-3}$ g./l. respectively, and the buffer concentration to 0·02 M (see Fig. 10).

●----● Heparin added with the platelets to a concentration of $1{\cdot}06 \times 10^{-3}$ g./l.

○----○ Dextran sulphate added with the platelets to a concentration of $1{\cdot}80 \times 10^{-3}$ g./l.

Taking, in Figure 11, the pair of curves shown in stipple (which represent the interference of the drugs with the reaction(s) of the preincubated mixture) the results suggested that, in the selected concentrations, heparin had by far the greater activity against reaction(s) not involving platelets. The other pair of curves show a much smaller difference.

As a simple rider to this experiment, clotting times were measured after recalcifying various mixtures of high spun and low spun citrated plasma (i.e. to obtain various platelet concentrations) containing different concentrations of the two drugs. One series of results with two platelet concentrations is illustrated in Figure 12. Each pair of points spans the same logarithmic interval of drug concentration, but it was necessary to stagger the pairs of heparin concentrations in order to keep the clotting times within the useful working range. For each drug, the curves from the different platelet concentrations are approximately parallel, but the heparin curves are separated by a greater vertical interval (about 0·035 reciprocal units, on the right hand scale) than those obtained with dextran sulphate (about 0·02 reciprocal units). In other words, a given reduction in the platelet count produces a greater prolongation of the clotting time in the presence of heparin than dextran sulphate. Now, the clotting time of recalcified, citrated plasma alone is relatively little affected by variations in the platelet count, provided that the plasma is allowed adequate contact with a "foreign" surface[21]. From this it may be inferred that reactions between factors other than platelets can to some extent replace platelet reactions in spontaneous clotting. The findings of Figure 12

thus suggest that dextran sulphate interferes less than heparin with reactions between non-platelet factors which confirms the inference drawn from Figures 10 and 11. Heparin has been shown to interfere more strongly with the thromboplastin generation test when fewer platelets are added to the reaction.[22].

(iii) *Effect of Dextran Sulphate on the reaction between Christmas Factor and Calcium*

Bergsagel[23] found that decalcified serum was less efficient in the thromboplastin generation test than untreated serum, and concluded that in serum, Christmas factor was combined with calcium; it appeared that a reaction between Christmas factor and calcium occurred at a preliminary stage in thromboplastin generation.

The effect of dextran sulphate upon this reaction was investigated as follows:—

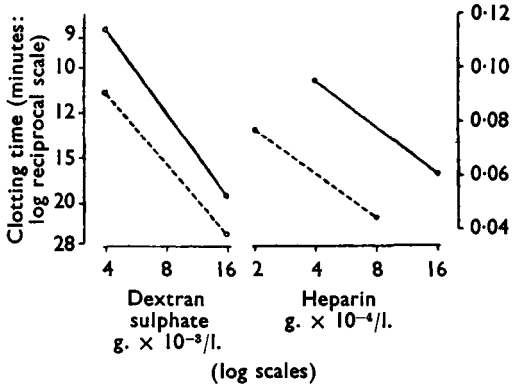

FIG. 12. *Part* 3: *Clotting times of recalcified citrated plasma.* Unbroken lines, platelets 170,000 per mm³. Broken lines, platelets 500,000 per mm³. International Standard heparin; British Standard dextran sulphate.

(1) A concentration of dextran sulphate was determined which was just insufficient to inhibit the thromboplastin generation system with untreated serum (alumina-treated plasma, 4 per cent.; serum, not previously diluted, 2·5 per cent.; British Standard dextran sulphate, $4{\cdot}0 \times 10^{-4}$ g./l.). When the tests were repeated with serum which had been decalcified by dialysis, this concentration of dextran sulphate produced a definite inhibition of thromboplastin generation.

(2) Dialysed serum, diluted overnight with saline, was incubated with calcium chloride solution and successive thromboplastin generation tests

TABLE I

REACTION BETWEEN CHRISTMAS FACTOR AND CALCIUM

Rates of activation of decalcified serum while being incubated with calcium chloride solution.

Dextran sulphate g./l.		Rate of Activation, per cent. per min. (replicates)		D-S-rate as per cent. of C-rate	
		Control (C)	Dextran sulphate (D-S)	Replicates	Mean
$4{\cdot}0 \times 10^{-4}$	1	4·1	1·9	46	61
	2	4·3	3·2	74	
	3	6·9	4·3	62	
$2{\cdot}0 \times 10^{-4}$		1·8	1·6, 2·5	89, 139	114

The method of computing the rate of activation is described in the text.

were made with subsamples of the incubating mixture. Thromboplastin generation became progressively more rapid until after about 40 minutes the results approximated to those obtained with previously diluted but undialysed serum. The time was noted at which each thromboplastin curve reached an indicator clotting time of 25 seconds, and as the serum was incubated, this clotting time was reached progressively more quickly. The log. of the time taken to reach 25 seconds was then plotted against the length of incubation of the serum, and the slope of this regression was expressed as per cent. increase in activity per minute incubation. Rates were also obtained with two concentrations of dextran sulphate and the results (Table I) suggested that the higher concentration ($4{\cdot}0 \times 10^{-4}$ g./l.) diminished the rate of reaction between Christmas factor and calcium. At half that concentration there was no apparent effect.

As the magnitudes of the effects were small, a comparison with heparin was not attempted.

These results suggest that the reaction between Christmas factor and calcium is rather more sensitive to dextran sulphate than is the generation of thromboplastin from active constituents. This goes some way towards explaining the discrepancy between the findings of Figures 8 (thromboplastin generation in whole blood) and 9 (thromboplastin generation from active constituents). Figure 9 showed a much greater difference between the minimal interfering concentrations of the two drugs. It may be that the activity of the two drugs is most similar upon the preliminary reaction(s) leading to thromboplastin generation, but that dextran sulphate has a lower activity upon all subsequent reaction(s).

Discussion

Dextran sulphate shows very different activities on the different phases of the clotting mechanism.

Tested upon the spontaneous coagulation of whole blood, as in the U.S.P. heparin assay[6], dextran sulphate was found to have approximately one-fifth of the activity of heparin[7].

The clotting time of whole blood largely depends upon the length of time taken in the earliest stages of coagulation[24]. It is therefore likely that when tests involving whole blood are used to assay an anticoagulant of the heparin type, the potency ratio will reflect the activity of the drug upon early reactions, and the present work confirms this hypothesis. The evidence further suggests that the actions of dextran sulphate differ so greatly from those of heparin that a true comparison between the anticoagulant activities of the two drugs cannot be made.

Summary

1. The anticoagulant activity of dextran sulphate has been compared with that of heparin. Compared with heparin, dextran sulphate did not greatly affect the thrombin-fibrinogen reaction, and reacted only slightly with the progressive antithrombin and the heparin co-factor, over the concentrations tested; an effect upon prothrombin conversion was not observed.

2. The relative effect of dextran sulphate upon thromboplastin generation was similar to the potency ratio derived from whole-blood clotting time assays.

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