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OBSERVATIONS ON THE EFFECTS OF A SERIES OF DEXTRAN SULPHATES OF VARYING MOLECULAR WEIGHT ON THE FORMED ELEMENTS OF THE BLOOD *IN VITRO*

BY

KENNETH W. WALTON

From the Department of Experimental Pathology, University of Birmingham

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The sulphuric esters of dextran, a complex polymer of glucose, show heparin-like anticoagulant activity (Grönwall, Ingelman, and Mosimann, 1945; Ingelman, 1947; Ricketts, 1952). In a previous communication (Walton, 1952) it was reported that differences of behaviour were demonstrable between members of a series of dextran sulphates prepared from parent dextrans of varying molecular weight. Although all the compounds behaved qualitatively like heparin in showing antithrombin activity, it was found that the compounds with molecular weights above a certain critical level caused, in addition, the precipitation from solution of fibrinogen (and possibly other macromolecular proteins) when mixed with plasma under physiological conditions. On the other hand, under similar conditions, the members of the series of dextran sulphates with molecular weights below this critical level did not interfere with the stability in solution of the plasma proteins.

It was found that the compounds which caused precipitation of the plasma-proteins also caused agglutination of the white-cells and blood platelets and produced alterations of the erythrocyte sedimentation rate. The compounds which did not precipitate the plasma-proteins produced no demonstrable alteration in the formed elements of the blood. The present communication presents details of the investigation of these differences in the reactions of the various blood cells to the dextran sulphates of large and of small molecular weight. The effect of various heparin samples on the formed elements of the blood was investigated simultaneously.

MATERIALS AND METHODS

Dextran Sulphates.—The compounds employed were selected members of the series previously described (Ricketts, 1952; Walton, 1952). They formed two main molecular weight groups:

Large Molecular Weight Group.—The compounds originally examined in this group ranged in molecular weight from 515,000 to 39,800. They were divided into three sub-groups: D (3 samples), of average molecular weight 458,000; A (2 samples), of average molecular weight 129,000; and E (2 samples), of average molecular weight 47,000. The samples within each sub-group originated from the same parent dextran, but differed in the extent of their sulphation. It was found that, in relation to their effects upon the blood cells, the compounds in any one sub-group produced qualitatively similar effects. For the sake of brevity, only the results obtained with the compounds of sub-groups D and E are reported. The middle sub-group, A, produced intermediate effects.

Small Molecular Weight Group.—This contained only one sub-group, I (3 samples), of average molecular weight 7,500.

Heparin.—Commercial heparin preparations were used for qualitative comparisons with the dextran sulphates. For most purposes, the preparation used was "Pularin" (Evans), batches No. F85020 and No. N10090, both assaying at 90 i.u. per mg. For more extended investigation of the effect of heparin upon platelets, the following additional preparations were employed: Heparin (Boots), batch No. 3583, 102 i.u. per mg.; "Liquemin" (Roche), batch dated 31.8.48, 122 i.u. per mg.; Heparin (British Drug Houses), batch No. 30607/c, 160 i.u. per mg.

Siliconed Glass-ware.—Two silicone preparations were employed: (1) "Teddol" (British Thomson-Houston Co. Ltd.), which is stated to be a mixture of methyl-chlorosilane and dimethyl-chlorosilane. A 1% solution in petroleum ether was applied repeatedly to the glass-ware, which was drained and allowed to dry in a fume-cupboard between applications. The vessels so treated were washed well with distilled water before use. (2) "DC 1107" (Albright and Wilson Ltd.) as a 3% solution in carbon tetrachloride was applied to the glass-ware, which was then drained and subsequently baked in an oven at 150° C. for fifteen minutes.

Both compounds were found to be effective in prolonging the coagulation time of the blood and in

preserving the platelets, but it was found to be essential to use perfectly clean, unscratched glass-ware and to ensure complete coating of the treated surfaces by repeated applications to obtain results comparable with those previously reported (Jaques, Fidlar, Felstead, and MacDonald, 1946; Patton, Ware, and Seegers, 1948).

Platelet-counting Methods.—Lempert's (1935) modification of Kristenson's (1922) method, and the method of Rees and Ecker (1923), were used. The latter method was modified by the substitution of 1% formalin for the 0.1% concentration originally suggested.

At high concentrations of heparin and of the dextran sulphates, it was found that the anticoagulants formed insoluble precipitates with the brilliant cresyl blue contained in both diluting fluids. For this reason, in some experiments, the dye was omitted and counting performed with the phase-contrast microscope.

Toluidine Blue.—Batch No. 851 of toluidine blue G (G. T. Gurr Ltd.) was used as a 1% (w/v) solution in distilled water.

RESULTS

Effect of Heparin and Dextran Sulphates on Whole Blood in vitro

It was reported previously (Walton, 1952) that the D and E size (large molecular weight) dextran sulphates caused precipitation of fibrinogen on addition to plasma or fibrinogen solutions. The precipitates formed, under physiological conditions of temperature, electrolyte concentration, etc., were found to be loose, easily dissociable complexes. Their formation was shown to be dependent primarily upon the molecular size rather than the content of sulphate groups of the acid polysaccharides. For this and other reasons the reaction was regarded as being due mainly to the interplay of non-ionic forces between the similarly-shaped asymmetrical molecules of the reactants. The effect of this interaction upon the stability of the formed elements of the blood was investigated in the following manner:

Experiment 1.—3 mg. each of heparin and of the D, E, and I size dextran sulphates was weighed out in siliconed glass tubes. A fifth tube contained the oxalate mixture of Heller and Paul (1934). 20 ml. of blood was withdrawn from the antecubital vein of a normal human subject, using a siliconed syringe and needle and with the adoption of the usual precautions to avoid contamination with tissue-juices. The first and last few ml. of blood in the syringe was discarded and the remainder was divided between the tubes containing the anticoagulants so that each tube contained 3 ml. of blood. The tubes were rotated gently to ensure solution of the anticoagulants in the blood. Thin smears (as for a differential white cell count) were prepared from the blood in each tube. Platelet-counts were then performed on each blood sample. Following this, the tubes were allowed to stand undisturbed for two hours, but were observed from time to time during this period. These observations showed that the rate and the final extent of sedimentation of the red-cells was approximately the same in the tubes containing oxalate, heparin, and the I size dextran sulphate, but was markedly less in the tubes containing the large molecular weight dextran sulphates. It was also noted that the plasma in the heparin, oxalate, and small molecular weight dextran sulphate tubes showed the shimmering opacity which is observed normally, while the plasma in the tubes containing the D and E size dextran sulphates was quite clear and transparent. Platelet-counts were now carried out on the plasma in each tube. The results are shown in Table I, from which it can be seen that there was good agreement between the platelet-counts in whole blood and in plasma treated with oxalate, heparin, and the I size dextran sulphate. On the other hand, the counts in the whole blood treated with the D and E size dextran sulphates were reduced while the counts in the corresponding plasmas (after sedimentation of the red-cells) were even more markedly diminished.

When the blood-smears made at the beginning of the experiment were stained with Leishman's or Giemsa's stain and examined microscopically, large basophilic masses were noted near the edges and in the "tails" of the smears made from the blood treated with the D and E size dextran sulphates. At the periphery of these masses individual platelets could be discerned, while the centres consisted of amorphous material in which large numbers of agglutinated platelets and occasional polymorph leucocytes were enmeshed (see Fig. 1).

Examination of the rest of the smear showed only an occasional discrete platelet here and there. Smears

TABLE I

EFFECT OF HEPARIN AND OF THE DIFFERENT DEXTRAN SULPHATES UPON PLATELETS IN WHOLE BLOOD *IN VITRO*, AS COMPARED WITH OXALATE

Compound	Platelet-Count*		Appearance of Blood-smear	Haematocrit (%)	Supernatant Plasma	Buffy Layer (%)
	Whole Blood	Plasma				
D size	36,000	< 10,000†	Basophilic clumps ++	38	Clear	2·0
E size	124,000	< 10,000†	,, ,,	38	,,	1·5
I size	336,000	310,000	Single platelets	38	Opalescent	1·0
Heparin	328,000	304,000	,, ,,	38	,,	1·0
Oxalate	352,000	308,000	,, ,,	38	,,	1·0

* Mean of three counts expressed as number per c.mm.
† Single platelets only counted. Many platelet clumps also present.

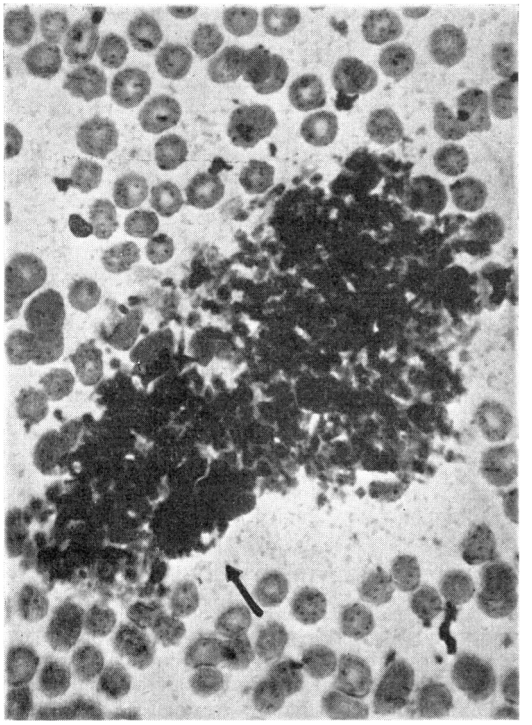

FIG. 1.—The effect of adding the D size dextran sulphate to whole blood *in vitro*. The blood-smear contains an irregular mass, consisting of a large number of platelets and a single leucocyte (marked by the arrow) clumped together. Leishman's stain. × 700.

made from the blood treated with oxalate, heparin, or the I size dextran sulphate showed the normal random distribution of single platelets with only occasional small platelet-clumps. These were free from basophilic material. No abnormal morphological changes were noted in the red or the unagglutinated white cells, in any of the smears.

Finally, the blood from each tube was transferred to a haematocrit tube and centrifuged at 3,000 r.p.m. for 30 minutes. As noted in Table I, the volume of packed red-cells in all the tubes was identical, but the tubes containing the large molecular weight dextran sulphates showed a thicker "buffy coat." When removed and examined microscopically, the buffy coat from the oxalate, heparin, and I size dextran sulphate tubes was found to consist of the usual mixture of large numbers of leucocytes and erythrocytes with a few small clumps of platelets and large numbers of single platelets. The buffy coat from the tubes containing the D and E size dextran sulphates consisted of leucocytes and erythrocytes in apparently normal numbers, but there were also present many shreds and loose flocculent masses of material to which platelets and leucocytes were adherent. Few discrete platelets were seen (see Fig. 2). When a drop of 0.1% brilliant cresyl blue was added to a hanging-drop preparation made from the buffy coat in the D or E size dextran sulphate tube, each flocculent mass was seen to consist of material staining a bright reddish-purple with large numbers of dark blue platelets clustered on the surface or interspersed among it.

From the results of this experiment, summarized in Table I, it appeared that the small molecular weight dextran sulphate, like heparin or oxalate, had had little or no effect on the formed elements of the blood. This variety of dextran sulphate had been shown previously not to affect the stability of the plasma-proteins. On the other hand, the large molecular weight dextran sulphates which had been found previously to form insoluble complexes with fibrinogen and other macromolecular plasma proteins, were now found to cause agglutination of platelets and white-cells. The behaviour of these compounds in relation to the individual formed elements of the blood was investigated further.

Effect of the Dextran Sulphates on Platelets

It was noted in Experiment 1 that in each platelet-clump found in the buffy coat of blood treated with the large molecular weight dextran sulphates,

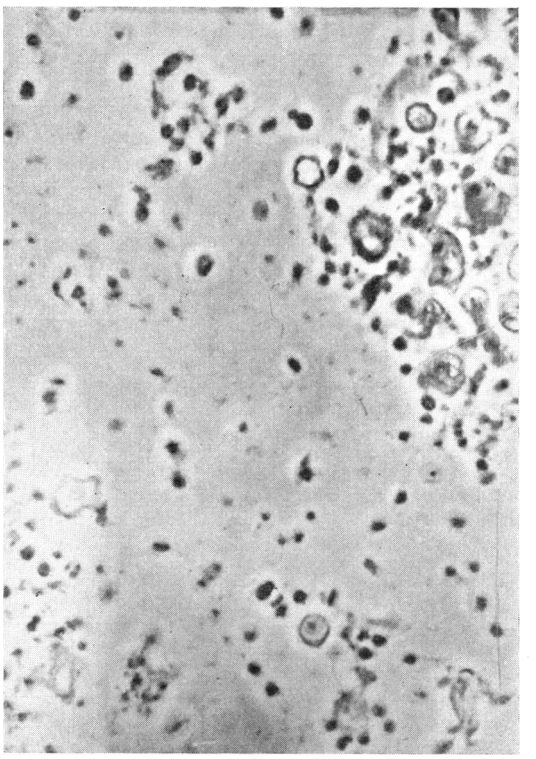

FIG. 2.—The effect of adding the E size dextran sulphate to plasma *in vitro*. A mass of agglutinated platelets and leucocytes is seen at the top right. Smaller clumps are present at the top centre and bottom right, while scattered single platelets are present elsewhere in the field. Unstained preparation photographed under the phase-contrast microscope. × 430.

the nidus of the clump consisted of amorphous material. This material was found to be strongly metachromatic when stained with cresyl blue. It was found that dextran sulphate alone or in combination with fibrinogen stained in this manner also. It therefore seemed reasonable to suppose that the clumping of the platelets occurred because of their adherence to the freshly formed surface of the complex or because the platelets acted as foci for the formation of the particles of the precipitated complex. In order to test these hypotheses the following experiment was undertaken:

Experiment 2.—A suspension of platelets in saline was prepared as follows: 20 ml. of blood was taken in a siliconed syringe from a normal human subject. The blood was placed in a cooled, siliconed glass tube and centrifuged at 0° C. and at 15,000 r.p.m. for 30 minutes. The plasma was removed and the buffy coat taken up in 2 ml. of 0.85% saline. This saline suspension was repeatedly centrifuged at 15,000 r.p.m. and washed in the cold with saline till a sample of the supernatant no longer gave a precipitate on the addition of a drop of 10% trichloracetic acid. The deposit was then judged to be free from plasma protein. It was resuspended in saline and examined under the microscope. It was found to consist of leucocytes, large numbers of single platelets, and many platelet-clumps. The suspension was then centrifuged lightly to remove the leucocytes and clumped platelets. The supernatant, consisting almost entirely of single platelets, was withdrawn. Five drops of this suspension were placed in each of four siliconed tubes, using a siliconed pipette. One drop of a 1% solution of heparin or of one of the dextran sulphates was then added to each tube. The tubes were shaken gently and one drop of each mixture in turn was placed on a siliconed microscope slide and examined under a phase-contrast microscope. In a second series of siliconed tubes, similar mixtures of the anticoagulants were made with the platelet suspension, but now to each tube there were added two drops of citrated plasma or serum. Again a drop of the final mixture from each tube was removed and examined. The contents of a control tube containing the platelet suspension mixed with plasma or serum alone were also examined.

It was found that the addition of heparin or of any one of the dextran sulphates to the saline suspension of platelets produced no alteration. But when serum or plasma was added to the mixture containing the large molecular weight dextran sulphates, the platelets could be seen to become entrained in, and adherent to, the precipitate which slowly formed, in a manner similar to that observed previously (see Fig. 2). The volume of precipitate and the amount of platelet-agglutination which occurred were both markedly greater with plasma than with serum. The addition of plasma or serum to the mixtures containing the I size dextran sulphate produced no precipitation of protein and the platelets remained unagglutinated.

In confirmation of the finding of Copley and Houlihan (1947) it was observed that certain samples of serum (whether homologous or heterologous in relation to the platelets) caused apparent agglutination of the saline suspension of platelets in the control tube. Such samples of serum were rejected. Further examination of these apparently spontaneously-formed clumps of platelets in certain samples of serum and plasma showed that gentle pressure on the cover-slip or microprobing with a very fine siliconed glass probe resulted in dispersal of the clump into its individual platelet elements. On the other hand, similar treatment of the much larger and more numerous clumps in serum or plasma containing the large molecular weight dextran sulphates did not result in similar dispersal. Instead, it was observed that, with the passage of time, the platelets present in the clumps slowly underwent granular disintegration so that the clump was eventually composed of amorphous material in which no separate elements were distinguishable. At a corresponding interval, the clumps formed in serum alone or in plasma containing citrate, oxalate, heparin, or the I size dextran sulphate were still composed of individually distinguishable platelets, and these could still be dispersed in the manner described above.

In other experiments various other particulate suspensions were substituted for the saline suspension of platelets. The materials used included a formolized suspension of *S. marcescens* in distilled water, watery suspensions of colloidal graphite varying in particle size from 0.5 μ to 3.0 μ, and a watery suspension of finely powdered kaolin (Crooke's collosal kaolin). In some experiments a 0.6% solution of human fibrinogen was substituted for the plasma or serum. It was found that the large molecular weight dextran sulphates did not affect the suspension-stability of any of these materials, in the absence of protein from the suspending medium. The addition of fibrinogen, plasma, or serum to mixtures containing the large molecular weight dextran sulphates produced agglutination of the particles with a corresponding order of efficiency. The small molecular weight dextran sulphate did not produce a similar effect.

Effect of the Dextran Sulphates on Erythrocytes

As shown in Experiment 1, none of the dextran sulphates affected the haematocrit value of blood to which it was added. It appeared, therefore, that these compounds, at the concentrations employed, exerted little or no osmotic effect on the red-cell. It was found that the presence of the I size dextran sulphate did not interfere with the ordinary haema-

tological investigations (red-cell count, haemoglobin estimation, etc.). The use of the I size dextran sulphate for keeping blood fluid for various laboratory procedures has been described by Hall and Ricketts (1952).

It is well known that the sedimentation rate of the erythrocytes is dependent upon rouleaux formation. It was first shown by Fåhraeus (1921) that alterations in the pattern of the plasma-proteins cause marked changes in the tendency to rouleaux formation and hence changes in the sedimentation rate. Increase of the macromolecular protein components, and especially an increased concentration of fibrinogen, the largest and most asymmetric of the normal plasma-proteins, results in acceleration of the sedimentation rate, under standardized conditions. It was shown by Ingelman and Halling (1949) that the addition to blood of macromolecular dextrans produced similar effects. This observation was confirmed and extended by Hardwicke, Ricketts, and Squire (1950), who also showed that other macromolecules were equally effective. They found that the phenomenon could be demonstrated most clearly after eliminating the effect of the normal sedimenting substance in plasma (fibrinogen) and after standardization of the red-cell volume at some given level (say 30% packed-cells). In such circumstances, increasing concentrations of any given macromolecule, with a molecular weight in excess of a certain critical level, produced corresponding increases in the sedimentation rate.

Since the large molecular weight dextran sulphates were known to precipitate the macromolecular proteins when added to plasma, it seemed likely that they would affect the sedimentation rate in a complex manner. Inhibition of sedimentation appeared likely to occur at concentrations sufficient to precipitate fibrinogen from whole blood. At higher concentrations, the "macromolecular effect" of the compounds themselves appeared likely to cause acceleration of sedimentation. It appeared probable that the small molecular weight dextran sulphates and heparin, which neither precipitated the plasma-proteins nor were large enough to produce direct effects, would fail to have any influence on the sedimentation rate. Experiments were designed to test these hypotheses.

Experiment 3.—In this experiment the various dextran sulphates and heparin were added in low concentrations to whole blood in which the concentration of fibrinogen and the sedimentation rate were known to be raised. The sedimentation rates of the samples containing the large molecular weight compounds were compared with that of a defibrinated sample of the same blood. The sedimentation rates of the samples containing heparin and the I size dextran sulphate were compared with an oxalated sample of the same blood.

Blood was taken from a patient suffering from an acute exacerbation of rheumatoid arthritis. The plasma contained 0.62 g.% of fibrinogen. 3 ml. aliquots of the blood were placed in each of a series of tubes containing 1 mg. of heparin or of the various dextran sulphates. A similar quantity of blood was added to a tube containing the balanced oxalate mixture of Heller and Paul. The blood was mixed well with the anticoagulants and transferred to Wintrobe tubes. Another sample of the same blood was defibrinated by constant stirring with a bent glass rod. The defibrinated blood was also placed in a Wintrobe tube. All the Wintrobe tubes were then suspended vertically in a water-bath at 25° C. and the fall of the column of red-cells was observed at five-minute intervals and plotted. The observed sedimentation rates (uncorrected) are shown in Fig. 3.

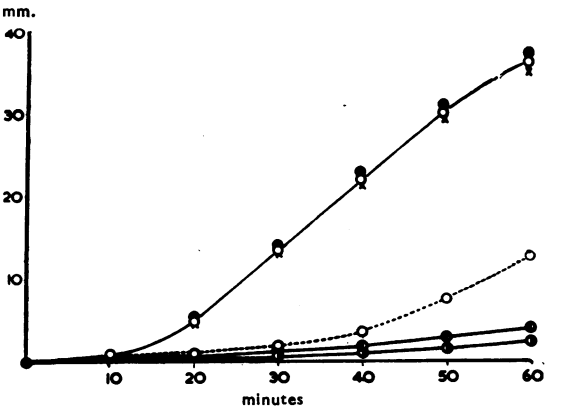

FIG. 3.—Effect on a pathologically raised erythrocyte sedimentation rate of the addition of the various anticoagulants as compared with the effect of defibrinating the same blood. X——X: Oxalate. ●——●: Heparin. ○——○: I size dextran sulphate. ○- - -○: E size dextran sulphate. ◐——◐: D size dextran sulphate. ◑——◑: Defibrinated blood.

It will be seen that there was close correspondence between the observed values for whole blood treated with oxalate, heparin, and the I size dextran sulphate. Practically no sedimentation occurred in the defibrinated blood or the blood treated with the D size dextran sulphate, while the curve for the E size compound (which showed less tendency to precipitate fibrinogen) occupied an intermediate position.

Experiment 4.—In this experiment the technique of Hardwicke *et al.* was used to investigate the sedimenting effect of high concentrations of the anticoagulants upon a standardized suspension of red-cells, in the absence of fibrinogen.

30 mg. each of heparin and of the three different dextran sulphates was weighed out in small glass bottles. A control bottle contained the normal amount (4.2 mg.) of the balanced oxalate mixture. 20 ml. of blood was taken from a normal human subject and defibrinated by constant stirring with a bent glass rod. The packed cell volume of the red-cells was adjusted to 30% by the addition of physiological saline and the diluted blood was distributed between the bottles. 3 ml. volumes of blood were added to the bottles containing heparin and the dextran sulphates, and 5 ml. was added to the oxalate bottle. Estimation of the sedimentation rate of the red-cells was carried out as in the previous experiment. The results obtained are shown in Fig. 4.

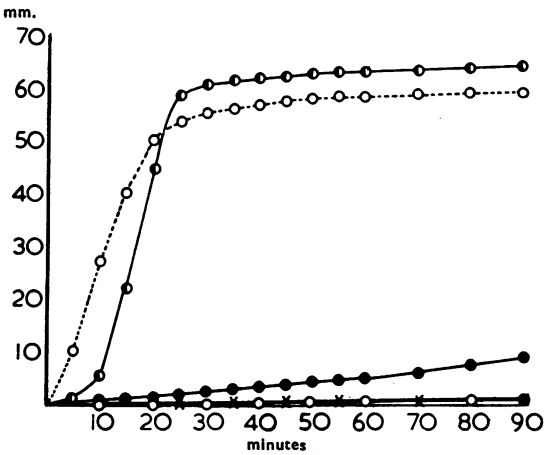

FIG. 4.—Sedimenting effect of the various anticoagulants on the erythrocytes in defibrinated blood. X——X: Oxalate. ●——●: Heparin. ○——○: I size dextran sulphate. ○---○: E size dextran sulphate. ◐——◐: D size dextran sulphate.

It will be observed that the sedimentation rate of the defibrinated blood containing 1% of the I size dextran sulphate did not differ significantly from that of the control containing the conventional concentration of balanced oxalate. The particular heparin sample used showed a slight sedimenting effect, but an enormously greater effect was obtained with the large molecular weight dextran sulphates. When the blood in the Wintrobe tubes was examined after sedimentation was complete, it was noted that rouleaux formation was markedly greater in amount in the tubes containing the D and E size dextran sulphates than in the control tube or in the tubes containing the I size dextran sulphate or heparin.

Effect of the Dextran Sulphates on Leucocytes

The effect of the dextran sulphates on the motility and phagocytic activity of leucocytes was investigated in the following manner:

Experiment 5.—Tenfold dilutions of heparin and of each of the dextran sulphates were made from stock solutions (containing 50 mg. per ml.) in 0.2 ml. volumes of mammalian Ringer solution. 0.8 ml. of blood, freshly drawn from a normal human subject, was added to each tube. In this way the final concentrations of heparin and of the dextran sulphates in the blood varied from 10 mg./ml. to 0.1 mg./ml. The tubes were incubated at 37° C. for fifteen minutes. At the end of this time thin smears and wet preparations were made from each tube. The wet preparations were examined immediately on a warmed stage under the phase-contrast microscope. Many leucocytes were observed to be entrained in the flocculent protein precipitates produced by the D and E size dextran sulphates, but the unagglutinated polymorphonuclear leucocytes and monocytes showed unimpaired motility at all concentrations of these compounds. Heparin and the I size dextran sulphate neither caused agglutination of the leucocytes nor affected their motility within the period investigated. When further samples were taken at intervals up to three hours, the leucocytes in the blood containing the dextran sulphates began to show the same degenerative changes that became noticeable in the blood kept fluid with heparin or oxalate. It was therefore evident that the dextran sulphates possessed no advantage over the latter as preservatives for the leucocytes.

Examination of the early stained films showed no morphological alterations of the leucocytes. The later films showed the presence in occasional monocytes of granules of basophilic material. This material stained metachromatically with toluidine blue. These cells were present in sufficient numbers to make it unlikely that they were degenerate basophilic leucocytes.

The experiment was repeated, but on this occasion two drops of a saline suspension of a coagulase-negative staphylococcus were added to each tube. The tubes were incubated at 37° C. for thirty minutes and once again smears and wet preparations were made. Examination of these showed that normal phagocytosis of the staphylococci had occurred at all concentrations of all the compounds.

Discussion of the in vitro *Effects of the Dextran Sulphates*

From the results of the experiments so far described, it is evident that the dextran sulphates, if of molecular dimensions greater than a certain critical limit, cause the aggregation of the formed elements of the blood. Earlier investigation of these large molecular weight compounds had shown that the particles of the insoluble complexes formed with fibrinogen tended to aggregate as floccules under suitable environmental conditions. Cohesion between the particles forming these floccules was assumed to be dependent upon weak intermolecular forces, since moderate thermal agitation of the molecules was sufficient to dissociate the floccules (Walton, 1952). Whether similar non-ionic forces would be adequate to bring about the association of the much larger formed elements of the blood is conjectural, but

there is some evidence that the agglutination of bacteria and of sensitized red-cells might be brought about by multiple non-ionic linkages resulting from antigen-antibody union at the cell-surfaces. It appears conceivable that the large molecular weight dextran sulphates might form complexes with the components of the plasma-protein film assumed to be present normally on the surface of platelets, erythrocytes, and leucocytes and thereby initiate conglutination of these structures in a similar manner.

Support for this hypothesis was provided by the observation that the large molecular weight dextran sulphates produced no agglutination of platelets, bacteria, or suspended particles of kaolin or graphite in the absence of protein in the suspending medium, but did bring about their aggregation on the addition of fibrinogen solution, plasma, or serum. Further support was provided by the observation that the small molecular weight dextran sulphate and heparin which did not form insoluble complexes with the plasma-proteins did not affect the stability of the formed elements of the blood.

The present findings are in keeping with those of Piper (1945a), who found that all the synthetic polysaccharide sulphates examined by him caused the agglutination of platelets. This was later associated with the fibrinogen-precipitating properties of the same compounds (Piper, 1945b). But in a subsequent communication Astrup and Piper (1946) implied that they were not entirely satisfied with this explanation, since they noted that an excess of cellulose trisulphuric acid or of chitin disulphuric acid still agglutinated platelets although visible precipitate formation was inhibited. On the other hand, in agreement with the present observations, they too noted that suspensions of kaolin or bacteria were only agglutinated in the presence of plasma.

It should be noted that although no precipitate is visible immediately with great excess of these compounds, yet it can be shown that interaction with fibrinogen still occurs, just as antigen-antibody union occurs in the " pro-zone " region of a precipitin titration, as shown by eventual visible precipitate formation even in this region (Walton, 1952). The suggested mechanism whereby particles become agglutinated by these compounds might still, therefore, be the same.

Complex formation with a plasma-protein fraction also occurs with heparin and with the I size dextran sulphate. Indeed the antithrombin activity of both compounds is dependent upon complex formation with a co-factor present in serum or plasma and suspected to be in the alpha-globulin or alpha-lipoprotein fraction. The complex formed with this fraction by either compound is, however, soluble under physiological conditions. Hence, it is suggested, its formation is not attended by any disturbance of the suspension stability of the plasma.

It has been reported by various authors that some samples of heparin do cause agglutination of platelets (Copley and Robb, 1941 ; Copley, 1948) and of leucocytes (Wilander, 1938). These observations were confirmed by Fidlar and Jaques (1948), who showed that carefully purified heparin did not behave in this manner. Quick, Shanberge, and Stefanini (1948) reported that the platelets in human blood were less sensitive to the agglutinating effect of heparin than the platelets in dog blood. Four preparations of British commercial heparin of varying degrees of purity (as judged by differences in anticoagulant activity) were examined personally, but failed to produce agglutination of the platelets in human blood. The heparins used were all stated to originate from beef lung. The investigation was not extended to include heparin derived from other tissue, nor was the effect on blood or blood cells from other species investigated.

It has been shown that purified heparin, at anticoagulant concentrations (1 mg. per 5–50 ml. blood), has no appreciable effect on the sedimentation rate (Wilander, 1938 ; Nielsen, 1942), but conflicting results were obtained with earlier crude preparations (Enocksson, Gjertz, Schnell, and Torgersruud, 1936) and with high concentrations (1 mg. or more per ml. blood) of commercial heparin (von Kaulla, 1939 ; Sappington and Gillis, 1941).

Even purified heparin is acknowledged to be polydisperse with regard to molecular size (O'Keef, Russo-Alesi, Dolliver, and Stiller, 1949). The molecular weight of 17,000 obtained by Grönwall *et al.* (1945) and by Jensen, Snellman, and Sylvén (1948) was calculated to be the average of the molecules present in the preparations. It has been shown by Jorpes and Gardell (1948) that a common contaminant of commercial heparins is chondroitin sulphate, a compound of considerably larger molecular weight than heparin. It is tempting to suggest that the presence, in amounts varying from one sample to the next, of contaminants or impurities of this sort might have brought about, by a mechanism similar to that put forward for the large molecular weight dextran sulphates, the conflicting and anomalous results referred to above.

It will be shown in a subsequent communication that the effects produced by the large molecular

weight dextran sulphates on the blood cells *in vitro* also occur *in vivo* in experimental animals and are largely responsible for the greater toxicity of the D and E size compounds as compared with the I size compound or heparin.

Summary

1. A comparison was made between heparin and a series of sulphuric esters prepared from dextrans of varying molecular weight, in respect of the effects of these anticoagulants on the formed elements of the blood.

2. The dextran sulphates were found to be divisible into two broad groups: (*a*) Compounds with molecular weights in excess of 40,000 which differed from heparin in causing platelet and white-cell agglutination and variable effects on the erythrocyte sedimentation rate. (*b*) Compounds with an average molecular weight of about 7,500 which resembled heparin in producing no appreciable effects on the blood cells.

3. The difference between the two groups of dextran sulphates were considered to be dependent ultimately upon differences in the nature and behaviour of the non-ionic complexes which they formed with certain of the plasma-proteins. The large molecular weight group formed complexes mainly with fibrinogen. The small molecular weight compounds, like heparin, formed complexes with an incompletely identified fraction of the plasma-proteins, suspected to be the alpha-globulin.

4. The complexes formed with fibrinogen by the large molecular weight dextran sulphates were insoluble under physiological conditions. The precipitation of fibrinogen in this manner was shown to interfere with the suspension stability of the formed elements in the blood. As a result, the platelets and white-cells were found to agglutinate and the red-cells to aggregate in rouleaux and clumps. The possible detailed mechanisms of these changes are discussed.

5. On the other hand, the complexes formed by the small molecular weight dextran sulphates were soluble in plasma under physiological conditions. No agglutination of the platelets or white-cells occurred and there was no appreciable effect on the erythrocyte sedimentation rate.

It is a pleasure once more to acknowledge the advice and assistance of my colleague, Dr. C. R. Ricketts, in various aspects of this investigation. I am grateful to Professor J. R. Squire for his encouragement, for his helpful criticisms, and for his assistance with the manuscript.

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