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INTERACTIONS BETWEEN DEXTRAN, FIBRINOGEN AND PLASMA MEMBRANES

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Abstract—FITC-labelled dextran has been used to show that there is no complex formation between fibrinogen and dextran and that the ability of dextran to reduce the flexibility of the erythrocyte does not require fibrinogen as an intermediary. Dextran was found to form a loose adherent coat on erythrocyte membranes. It was also found to adhere much more strongly to platelet membranes. It is suggested that this may affect platelet function and be a cause of the haemostatic disturbances sometimes observed after dextran infusion.

The polysaccharide, dextran, has found common use in clinical medicine as a plasma volume expander and to improve blood flow [1]. In conditions of high shear rate the flow-improving property of dextran infusions is primarily the result of the plasma volume expansion [2, 3]. At low shear rates erythrocyte rouleaux and aggregates can form and their presence has a substantial influence on the viscosity of blood [4–6]. Thus the ability of some dextran preparations to break up rouleaux and aggregates [6, 7] can have a beneficial effect in this region. However, it is not yet clear whether these effects result from a direct interaction of dextran with the erythrocyte membrane, though there is evidence to suggest that it adheres to plasma membranes [8], or whether they are due to an indirect effect resulting from its interaction with fibrinogen. The second possibility must be considered because fibrinogen is known to have a very profound effect on red blood cell flexibility and rouleaux formation [7, 9] and to be precipitated by dextran [10].

In this investigation fluoresceine-labelled dextran has been used to confirm that dextran binds to erythrocyte membranes and to investigate the possibility of it complexing with fibrinogen. This latter aspect is not only of significance to haemorheology but also to haemostasis because there are many reports of dextran affecting the haemostatic mechanism *in vivo* and *in vitro* [11–14] and there is considerable evidence to show that it is a useful antithrombotic agent [15, 16].

MATERIALS AND METHODS

Blood. The blood used in these experiments was either collected with heparin ($12\frac{1}{2}$ i.u./ml) as anticoagulant or was defibrinated by stirring with a glass rod. In some experiments the blood was washed with Ringer–Locke solution (8·0 g NaCl, 0·2 g $NaHCO_3$, 0·42 g KCl, 0·24 g $CaCl_2$, made up to 1 litre with distilled water), by alternate centrifugation and removal of the supernatant in order to remove all plasma protein, the cells finally being suspended in the Ringer–Locke solution.

Fibrinogen. This was obtained in solid form from A. B. Kabi (Stockholm, Sweden). Solutions were made by dissolving it in distilled water rather than saline or Ringer–Locke solution since the solid contained citrate and NaCl.

Dextran. In order to be able to differentiate between fibrinogen and dextran when they were run simultaneously through gel filtration columns, the dextran used was tagged with a fluorescent label. This labelled material was obtained from Pharmacia G.B. Ltd. (London, W5) and was fluoresceinylthiocarbomoyl-dextran (FITC-dextran) with a weight-averaged molecular weight ($\bar{M}_w$) of 40,100.

Gel filtration

Sepharose 4B or Sephadex G200 (Pharmacia G.B. Ltd., London, W5) were used as the filtration media, in a column of length 0·5 m and diameter 2·5 cm. The elution media were either 0·85% (w/v) NaCl solution or Sorensen's pH 7·4 buffer [17]. The protein elution peak was determined by absorption at 280 nm. At this wavelength dextran has a very low absorption coefficient so its elution peak was obtained by collecting the elutant fractions and determining the fluorescence at 520 nm caused by excitation at 435 nm.

RESULTS

Membrane-binding. Small quantities of a solution of FITC-dextran in Ringer–Locke solution were added to heparinized blood, defibrinated blood, washed defibrinated blood to which fibrinogen had been added, and washed heparinized blood. A sample of each of these preparations was viewed under a fluorescence microscope: no differences were observed between them and the erythrocytes fluoresced strongly. The preparations were then repeatedly washed with Ringer–Locke solution and after each washing a sample from each preparation was viewed under the fluorescence microscope. Again there was no difference between the preparations for a given number of washes, and as expected the fluorescence of the red cells quickly diminished as the washes removed more and more of the FITC-dextran. However, the unexpected discovery was made that the platelets still fluoresced very strongly. Even after six washes, when the red cells exhibited little or no fluorescence, the platelets still shone very brightly.

Gel filtration. Approximately equimolar volumes of FITC-dextran in eluant solution and of fibrinogen

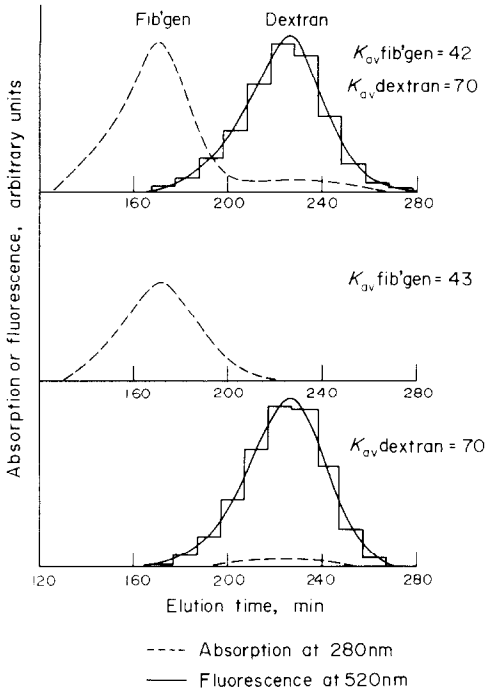

Fig. 1. The elution peaks of fibrinogen and FITC-dextran when run together or individually through a Sepharose 4B column.

solution were mixed and then passed through the gel filtration column. The absorption peak of the fibrinogen and the fluorescence peak of the FITC-dextran produced in this way were compared with the peaks produced by passing the fibrinogen or FITC-dextran separately through the column. Figure 1 shows typical results. There was no significant difference in elution volumes in either case. This was so irrespective of whether the eluant was saline or Sorensen's buffer or whether the filtration medium was Sephadex G200 or Sepharose 4B.

CONCLUSION

In a previous investigation Rampling [18] showed that the precipitation of fibrinogen by dextran was due to a non-specific physical effect of steric exclusion at high concentrations of dextran polymers. However, that investigation did not rule out the possibility of some complex formation between the fibrinogen and dextran. The gel filtration results reported above clarify the situation. The fact that there is no difference in the elution volumes of the fibrinogen and dextran when subjected to gel filtration individually or when mixed proves that no significant complex formation between the fibrinogen and dextran takes place. It would therefore appear that any effects produced on the haemostatic mechanism *in vivo* by infusions of dextran, and particularly the drop in fibrinogen concentration which often takes place [11], must result from causes other than complex formation with fibrinogen.

Previous investigations have shown that fibrinogen has a direct and profound effect on the flexibility of erythrocytes [7, 19]. It has also been shown that the addition of dextran to whole blood *in vitro* affects this flexibility [7]. The present investigation, in showing that dextran adheres to erythrocyte membranes, that the adherence is independent of the presence of fibrinogen and that there is no direct interaction between fibrinogen and dextran, makes it clear that the effect of dextran on erythrocyte flexibility must be a direct one – that is to say the binding of dextran to the erythrocyte membrane must of itself be able to cause flexibility changes, possibly as a result of alterations in the membrane structure. In this latter context it is significant that it has recently been shown that dextran can enhance cellular fusion caused by a variety of agents [20].

An unexpected finding of this investigation of dextran was its very strong binding to platelets. Since the binding to erythrocytes was less powerful but yet able to affect the mechanical properties of the erythrocyte, it is very likely that the mechanical properties of the platelet will also be affected. Furthermore, since the physiological function of platelets i.e. aggregation and surface adhesion, necessitate morphological changes, it is possible that the surface binding of dextran could affect this also. It is interesting to note in this respect that platelet adhesion and aggregation have been reported to be depressed in subjects who have been infused with dextran [12, 21].

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