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# A Basic Derivative of Dextran and its Interaction with Serum Albumin

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The esters of dextran with sulphuric acid provide electronegatively charged macromolecules with various molecular weights and numbers of charged groups (Ricketts, 1952). Their interactions with blood-plasma proteins have shown several interesting features, including anticoagulant activity (Walton, 1951) and the formation of insoluble complexes with fibrinogen (Walton, 1952), which was utilized in the purification of antihaemophilic globulin (Walton & Ellis, 1956). Similarly, insoluble complexes are formed with β-lipoproteins, enabling these proteins to be separated quickly from serum without atmospheric oxidation of the lipids (Oncley, Walton & Cornwell, 1957). These findings encouraged exploration of the interaction of electropositively charged dextran derivatives with plasma proteins. Such dextran derivatives were not already known but some insoluble diethylaminoethyl ethers of cellulose were prepared by Peterson & Sober (1956) for use as anion exchangers in protein chromatography. This paper describes the preparation and characterization of some water-soluble diethylaminoethyl ethers of dextran (basic dextrans, Fig. 1) and describes some features of their interaction with a typical soluble protein, serum albumin. A preliminary report of part of this work has already appeared (McKernan & Ricketts, 1959).

## MATERIALS

Four samples of dextran were used: the native dextran of *Leuconostoc mesenteroides* B. 512 with intrinsic viscosity 1·15 and three partially hydrolysed dextrans with intrinsic viscosities of 0·32, 0·10 and 0·04. The 2-chlorotriethylamine hydrochloride (Eastman Kodak Co., P. 6436) was recrystallized from methanol. Serum albumin was obtained from the Lister Institute.

## METHODS

*Preparation of basic dextrans.* Dextran (6 g.) dissolved in sodium hydroxide solution (4 g. of sodium hydroxide in 17 ml. of water) was cooled to 0° and 2-chlorotriethylamine hydrochloride solution (3·5 g. in 4·5 ml. of water) was added with efficient stirring. The temperature was raised to 80–85° for 35 min. After cooling, sufficient ethanol was added, with stirring, to precipitate completely the basic dextran, which was separated by centrifuging. The precipitate was dissolved in water and reprecipitated several times until the supernatant solution was colourless. A solution of the final precipitate was neutralized with hydrochloric acid, dialysed and concentrated under reduced pressure. The basic dextran was isolated as its hydrochloride by freeze-drying. From the four dextran samples, products containing up to 1·77% of N were obtained, as shown in Table 1. Products with higher N content were obtained by using more concentrated solutions, namely dextran (6 g. in 8 ml. of water) mixed with sodium hydroxide (4 g. in 4 ml. of water) with 2-chlorotriethylamine hydrochloride (6 g.) added as solid. The temperature of

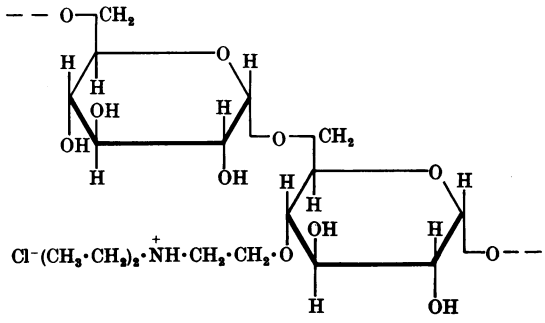


Fig. 1. Formula of basic dextran showing two glucose units forming part of the dextran molecule with a basic group, the diethylaminoethyl group, substituted at an arbitrarily chosen position. The hydrochloride salt of the base is shown.

Table 1. *Diethylaminoethyl ethers of dextran*

| Dextran intrinsic viscosity | Serial no. | N (%) | N (m-equiv./g.) | Cl (m-equiv./g.) | Basic group/ glucose unit | p*K* in water | Percentage of albumin precipitated (see text) |
|---|---|---|---|---|---|---|---|
| 0·04 | A | 1·05 | 0·75 | — | 0·16 | 8·7 | 2 |
| 0·10 | B | 1·77 | 1·26 | 1·23 | 0·26 | 8·8 | 7 |
| 0·32 | C | 1·23 | 0·88 | 0·90 | 0·18 | 8·7 | 12 |
| 1·15 | D | 0·98 | 0·70 | 0·66 | 0·14 | 8·9 | 35 |
| 0·04 | E | 3·47 | 2·48 | — | 0·64 | 8·9 | 4 |
| 0·10 | F | 4·00 | 2·86 | 2·79 | 0·80 | 8·8 | 29 |
| 0·32 | G | 3·33 | 2·36 | 2·29 | 0·60 | 8·5 | 51 |
| 1·15 | H | 2·22 | 1·59 | 1·51 | 0·34 | 8·9 | 80 |

80–85° was maintained for 4 hr. subsequently. The basic dextran was isolated as described above.

*Nitrogen.* This was determined by the micro-Kjeldahl procedure with selenium catalyst.

*Chloride.* This was determined by addition of excess of silver nitrate and titration with thiocyanate.

*Titration curve.* A portion (10 ml.) of a 1% solution of the basic dextran was passed through a column (11·5 cm. × 1 cm. diam.) of Amberlite IRA-400 resin to exchange chloride for hydroxide. The column was washed with water until the effluent was neutral; the solution was adjusted to a definite volume. A portion of the solution, stirred magnetically, was titrated with hydrochloric acid and the pH after each addition was measured with a Cambridge pH meter. Where stated, a lithium glass electrode was used to minimize errors in alkaline solutions and the effluent was collected and titrated under nitrogen to exclude carbon dioxide.

*Albumin concentration.* This was determined from the absorption at 280 mμ with a Unicam SP. 500 spectrophotometer. Basic dextran showed some absorption at this wavelength, e.g. preparation H showed $E^{1\%}_{1\,cm.}$ 0·390, which may be compared with $E^{1\%}_{1\,cm.}$ 6·6 for human-serum albumin. The basic dextran showed no definite absorption maximum. In the experiments described the contribution of the basic dextran to extinction has been ignored and it was calculated that the error so incurred was always less than 6%.

## RESULTS

In Table 1 the intrinsic viscosity of the dextran is shown as an indication of the molecular weight of each preparation. The basic dextrans were isolated in amounts ranging from 48 to 68% of the theoretical yield, with the exception of the two preparations of lowest molecular weight, for which the yield was approx. 14%, probably because of losses during dialysis and precipitation; the theoretical yields were calculated from the N content, assuming a structural formula similar to that shown in Fig. 1. The chloride content showed good agreement with the N content, as shown in Table 1. The average number of basic groups per glucose unit was also calculated from the N content. The reciprocal of this figure indicates the interval between basic groups, e.g. for preparation H there is, on average, one basic group to every three glucose units of the dextran molecule. Fig. 2 shows the titration curve for preparation B; the curves for the other preparations were similar. The inflexion in the region of pH 6 was at first attributed to carbon dioxide absorbed during stirring of the titration solution, cf. Peterson & Sober (1956). However, when the experiment was repeated under carbon dioxide-free conditions the inflexion persisted. A possible explanation is the presence of carboxyl groups (cf. Peterson & Sober, 1956; Porath, 1957), but dextran having 1:6 linkages could form carboxyl groups only at molecular-chain endings. A plot of $\Delta pH/\Delta v$ against $v$ enabled the end-point of the titration to be determined and hence the pH at half-neutralization. The p$K$ value showed no systematic variation with N content. The mean of eight p$K$ values (see Table 1), so obtained, was 8·8, and this value may be compared with pH 9·90 for diethylaminoethanol. With the lithium glass electrode an almost identical titration curve was obtained from which a p$K$ value of 8·85 for basic dextran preparation B was calculated.

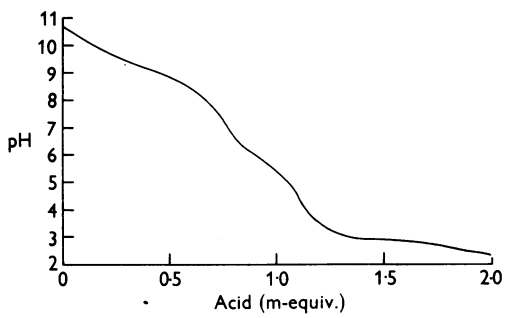


Fig. 2. Titration curve of basic dextran B in water.

### *Interaction of basic dextran with serum albumin*

Mixtures of albumin and basic dextran in buffered solution at pH 6·8 (Ostling & Virtama, 1946) produced only slight precipitates, which separated as a viscous liquid phase. In the absence of buffer ions flocculent precipitates were obtained, suggesting that precipitation was inhibited by the presence of buffer ions. Subsequent experiments were therefore conducted in the absence of buffer salts.

The precipitation of albumin by various basic dextrans was investigated in the following experiment: 1 ml. of 0·5% basic dextran solution was mixed with 3 ml. of 0·5% albumin solution and the pH was adjusted to 8·6 with sodium hydroxide. The control contained the same volume of albumin solution similarly adjusted to pH 8·6. After centrifuging, the albumin concentration in the supernatant solutions was determined spectrophotometrically. The percentage of the albumin precipitated is listed in Table 1. The figures show that precipitation of albumin increases with electrical charge and molecular weight of the basic dextran. The greatest precipitation was obtained with basic dextran H and this preparation was used to test the effects of variations in pH and electrolyte concentration.

The effect of variation of pH was studied at a constant albumin to basic dextran ratio of 2·5:1 in the presence of varying amounts of electrolyte. The results are shown in Fig. 3; it can be seen that the precipitation of albumin is extremely sensitive both to electrolyte concentration and to pH. In the absence of electrolyte (other than the NaOH

added to bring the solutions to the desired pH) three regions may be distinguished: (1) below pH 7, where no precipitation occurred whatever the concentration ratio of the reactants; (2) from pH 7 to about pH 9, where precipitation increased with increasing pH, reaching a maximum at about pH 8·5–9·0; (3) at pH values greater than 9, where precipitation decreased with increasing pH until about pH 11, beyond which no precipitation was observed.

Addition of sodium chloride or sodium thiocyanate to a final concentration of 4 mN caused a striking shift of the three pH regions toward lower values and diminished the amount of albumin precipitated under maximal conditions. Sodium thiocyanate was slightly more effective than sodium chloride at this concentration in bringing about these effects. Further increase of electrolyte concentration to 0·04N further enlarged these effects.

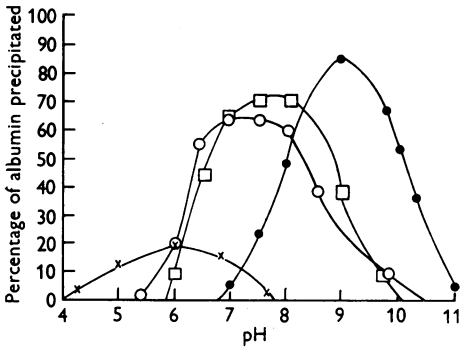


Fig. 3. Precipitation of serum albumin by basic dextran H under various conditions. Mixtures containing albumin and basic dextran in a constant ratio of 2·5:1 were adjusted to various pH values with HCl or NaOH. ●, No further electrolyte added; □, 4 mN-NaCl; ○, 4 mN-NaSCN; ×, 0·04N-NaCl.

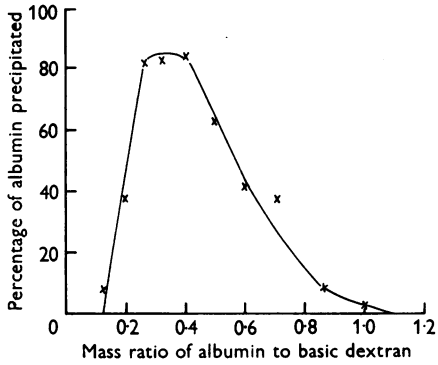


Fig. 4. Effect of varying the relative proportions of basic dextran and human-serum albumin under the optimum conditions for precipitation, namely pH 8·6 without added electrolyte.

In the presence of 0·04N-sodium chloride only 20% of the albumin was precipitated under maximal conditions and with 0·04N-sodium thiocyanate no precipitation was observed.

The effect of variation in the ratio of the reactants is shown in Fig. 4, where the percentage of albumin precipitated is plotted against the mass ratio of albumin to basic dextran. Three regions may be again discerned: (1) at mass ratios of albumin to basic dextran less than 0·25 basic dextran was present in excess and albumin precipitation was incomplete; (2) from ratios of 0·25–0·41 maximum precipitation was observed, about 85% of the albumin being precipitated; (3) at ratios exceeding 0·41 albumin was present in excess and precipitation was again incomplete.

## DISCUSSION

The highest degree of substitution obtained for basic dextrans, 0·8 group/glucose unit, is much lower than for dextran sulphate, in which 1·7 sulphate groups/glucose unit is readily obtainable. Nevertheless the efficiency of the procedure is reasonably good. With the proportions of reagents described by Peterson & Sober (1956) 23–45% of the amine was incorporated into the products. The incorporation of amine was improved to 34–70% by using more concentrated solutions of reagents and a higher proportion of amine. It is possible that repetition of the reaction would lead to higher degrees of substitution. The free base obtained by ion exchange appeared to be stable in solution, unlike dextran sulphate in which the free acid undergoes autohydrolysis. In the neutral hydrochloride isolated the equivalence of nitrogen and chloride is satisfactory. Experiments on hydrolysates of basic dextran have indicated that substitution occurs at the three available positions of the pyranose ring (McKernan, 1958). The mean p$K$ of 8·8 is similar to the value for the insoluble cellulose derivatives.

The precipitation of albumin has been shown to increase with increasing molecular weight and electropositive charge on the basic dextran, presumably because of the greater surface area of the larger basic dextran molecules, which allows more charged groups on the dextran to combine with oppositely charged groups on the protein. This is in agreement with the precipitation of fibrinogen by basic dextrans (McKernan, 1958) and by dextran sulphate (Walton, 1952). The precipitation of albumin at low salt concentrations by basic dextrans differs from the precipitation of fibrinogen, however, in that with the latter protein precipitation occurs on either side of the isoelectric point (pH 5·2) but with albumin precipitation occurs only above pH 7·0. Similar results have been

reported by several workers, for instance Morawetz & Hughes (1952).

The effect of pH and electrolyte concentration on the system is most marked (Fig. 3). In the absence of salt, precipitation does not occur below pH 7·0 but the addition of $Cl^-$ or $SCN^-$ ions shifts the pH range over which precipitation occurs towards lower pH values and decreases the amount of precipitate. The observation that thiocyanate is more effective than chloride in lowering the pH range of precipitation is in agreement with the results of Morawetz & Hughes (1952) for the precipitation of bovine-serum albumin by polyvinylamine.

The proportion of polycation to that of albumin was found to be an important factor in the precipitation studies (Fig. 4). Maximum precipitation was generally found to occur at a critical ratio of albumin to basic dextran. Above and below this ratio solubilization of the precipitate was produced—an effect similar to that obtained in an antigen–antibody-precipitin reaction.

The basic dextrans showed little effect on either the one-stage prothrombin time or the calcium clotting time. They formed insoluble salts with heparin and dextran sulphate as protamine does, so that neutralization of anticoagulant activity may be expected. It was noticed that basic dextran agglutinated suspensions of cells, e.g. *Escherichia coli*, *Staphylococcus aureus*, erythrocytes. Low concentrations of the high-molecular-weight preparations, e.g. 5–50 μg./ml. of preparation D, were most effective. The agglutinating effect is presumably due to the fact that these cell suspensions are stabilized by electronegative charges in the cell surface which are neutralized by combination with basic dextran molecules.


## SUMMARY

1. The preparation of dextran derivatives having an electropositive charge as opposed to the electronegative charge of dextran sulphates is described.

2. Some preliminary experiments on their interaction with proteins and cells are reported. The effect of pH, electrolyte concentration and the relative proportions of reactants on the formation of insoluble complexes with human-serum albumin were investigated. The optimum proportion of albumin to basic dextran lies within the range 0·256–0·41 by weight. At pH 8·6 without added electrolyte 85% of the albumin is precipitated.

3. Suspensions of erythrocytes and bacteria were agglutinated by basic dextrans.


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# Studies on Synovial Tissue

## 7. CONCENTRATIONS OF CREATINE AND PHOSPHOCREATINE IN SYNOVIAL TISSUE*


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Dingle & Thomas (1956) showed that the synovial proliferation which takes place in joints affected by rheumatoid arthritis is accompanied by a marked rise in metabolic activity of the affected tissue. In recent years, investigations into the biochemical mechanisms which control the rates of metabolic processes have emphasized the part played by phosphate and phosphate acceptors (Lardy & Wellman, 1952; Krebs, 1957; Kornberg & Krebs, 1957), and it was therefore considered that a study of the levels of energy-supplying phosphates and phosphate acceptors in normal and proliferating synovial tissue would further clarify the metabolic changes induced by the disease

* Part 6: Thomas, Dingle & Cook (1960).