The Interaction of Dextran with Serum Albumin, Gamma Globulin, and Fibrinogen

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ABSTRACT The interaction between dextran and serum albumin, gamma globulin, and fibrinogen can be studied by an electrophoretic method, which depends on obtaining electrophoretic patterns, first of each protein, then of dextran, and finally of mixtures of each protein with dextran. The areas under the electrophoretic spikes for each protein, for dextran, and for the mixtures are measured.

At pH's between 9.6 and 6.6, there is a transference of albumin to dextran when the two components are mixed, the amount of albumin lost being nearly equal to the gain in the new component, albumin plus dextran. This new species has a specific refractive index of about 0.00205 and seems to be composed of about one albumin molecule for every four dextran molecules.

The method is unsuitable for studying interaction between dextran and gamma globulin because these substances are almost immobile and do not separate into two spikes.

The method shows that a mixture of dextran and fibrinogen gives only one slowly moving spike (pH 6.6 to 8.6), the area under which is the sum of the areas under the dextran and the fibrinogen spikes taken separately. Either there is no interaction, or the new species has virtually the same refractive index increment as fibrinogen and dextran taken separately (0.0014 to 0.0015).

Carbone, Furth, Scott, and Crosby (1954) observed that there may be an interference with the hemostatic mechanism after the injection of dextran. It was later shown that platelets and red cells (Rothman, Adelson, Schwebel, Langdell, and Fraction (1957); Ponder (1957)) are coated by dextran, and recently that white cells are coated also. The question now arises as to whether dextran coats or, more generally speaking, interacts with isolated proteins.[1] This paper is concerned with the interaction between dextran and serum albumin (Cohn's fraction V) and with some observations on the interaction,

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[1] Seegers, Levine, and Johnson (1955) have already shown that dextran interferes with prothrombin activation.

or absence of interaction, of dextran with gamma globulin and fibrinogen. The method used for determining interaction between a protein and dextran depends upon the obtaining of electrophoretic patterns, first of the protein, then of the dextran, and finally of mixtures of protein and dextran, the areas under the electrophoretic spikes for the protein, for dextran, and the areas under the spikes for the mixtures being measured.

Material and Methods

The material used in these experiments must be reasonably monodisperse. The dextran was kindly supplied by Commercial Solvents Corporation in a 6 per cent solution and has an average molecular weight of 77,100 ± 20 per cent with a high fraction (10.1 per cent) not exceeding 168,400 and a low fraction (9.98 per cent) of 28,400 (lot 285W3A). The serum albumin, provided by the American National Red Cross, is about as monodisperse and has a molecular weight of 85,000. The samples of fibrinogen were provided by Dr. Walter Seegers and Dr. Kent D. Miller. The molecular weight is between 330,000 and 430,000 (Seegers (1955)). The gamma globulin, provided by Dr. Robert B. Pennell, has a molecular weight of about 300,000.

The electrophoretic mobility of the various components and their mixtures was measured with the Antweiler microelectrophoresis apparatus fitted with a Svensson-Philpot attachment and a camera, by means of which the electrophoretic pattern can be both observed as it develops and photographed. Patterns of the proteins, the dextran, and of their mixtures can be obtained and photographed at different pH's by using different buffers, and the areas under the spikes can be measured, after enlargement by projection, either by planimetry or by cutting out and weighing them.

The dextran in a concentration of 6 per cent[2] is diluted with Michaelis buffer at pH 9.6, 8.8, 8.0, or 6.6; these solutions are dialyzed against the appropriate buffers. Thereafter, the dextrans are diluted to give concentrations of 2.0, 1.5, 1.0, and 0.5 per cent. If the areas under the spikes are plotted against these concentrations of dextran, a straight line passing through the origin results. Similarly, albumin is dissolved in buffers at the same four pH's in a concentration of 4 per cent and dialyzed against the appropriate buffers; thereafter, it is diluted with the buffers to give concentrations of 2.0, 1.5, 1.0, and 0.5 per cent. If the areas under the spikes which develop are plotted against the concentration of dissolved albumin, another straight line results. Finally, mixtures of dextran and albumin are prepared by adding known

[2] While the experiments and results described in this paper are for systems in Michaelis buffer of constant ionic strength and of pH 9.6 to 6.6, essentially the same values have been found in systems made up in Dole buffer at pH 8.8 as in the systems containing Michaelis buffer. Michaelis buffer of constant ionic strength is described by H. B. Bull in Physical Biochemistry, (New York, John Wylie & Sons, Inc., 1943), and the directions for making it up are as follows: 9.714 gm. of sodium acetate (containing 3 molecules of water of hydration) and 14.714 gm. of the sodium salt of veronal are dissolved in water and made up to 500 cc. To each 5 ml. of this solution are added 2 ml. of an 8.5 per cent sodium chloride solution plus a ml. of 0.1 N hydrochloric acid and $(18 - a)$ ml. of water. The range of the buffer is from pH 9.64 ($a = 0.0$) to pH 2.62 ($a = 15.0$). The ionic strength is constant and is the same as that of human blood.

amounts of the dialyzed dextran and the dialyzed albumin at the same four pH's, the albumin concentrations in the mixtures being 2.0 per cent, 1.5 per cent, 1.0 per cent, and 0.5 per cent and the dextran concentrations being 2.0, 1.5, 1.0, and 0.5 per cent. Two spikes now develop in the electrophoresis pattern, the area under each of which can be measured. If the areas under the spikes are plotted against the concentration, first of protein in the mixture and then of dextran in the mixture, two straight lines result. For the sake of uniformity, photographs of the patterns are taken 6 minutes after the current is turned on. In this time, dextran moves very slowly from the junction of the channels, whereas albumin moves quite rapidly. (See Fig. 1.)

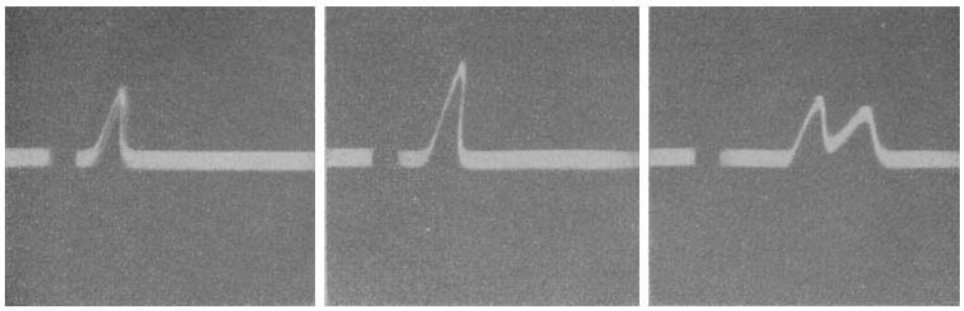

FIGURE 1. Electrophoretic patterns. Left to right, dextran spike, albumin spike, and the two spikes observed in the mixture. The curves are normalized for field strength and for time before the areas under the spikes are measured. Concentration of both dextran and albumin, 1 per cent.

The result is that four straight lines of different slopes, all passing through the origin, are obtained at pH 9.6, 8.8, 8.0, and 6.6. The slope of each line is K, which is approximately the same as α, the specific refractive increment, multiplied by instrumental constants. To avoid these constants changing, the slits of the electrophoresis apparatus are fixed. The slopes of the four lines give values of K at each pH, one for dextran, one (proportional to $\alpha = 0.0018$) for albumin, and two for the dextran spike and for the albumin spike in the albumin-dextran mixture.

Ideal electrophoresis is assumed throughout except for the interaction, and only the patterns of rising boundaries are used because the Antweiler microelectrophoresis apparatus is not suitable for the measurement of descending boundaries. The δ-boundary is scarcely visible in the Antweiler apparatus, and it has been neglected because of the assumption that the electrophoresis is ideal.

1. Interaction of Dextran with Albumin

The values of K can be measured (see Fig. 1 for the electrophoretic patterns of the albumin, the dextran, and the mixture, all at pH 8.0, and Fig. 2 for an example of the way in which the data are treated), and the change in area

under the dextran spike, as well as the change in area under the albumin spike (both in the mixtures), can be calculated by subtracting the K for the albumin in the mixture from the K for the albumin alone, and by subtracting the K for dextran alone from the K for the dextran in the mixture. Averaging the results at the four pH's, the gain in area under the dextran spike in the mixture is +12.0, while the loss of area under the albumin spike in the mixture is −11.5. (The example in Fig. 2 gives +11.0 and −10.0.) From this it can be concluded that there is a transference of albumin to dextran when the two components are mixed together and that the amount of albumin lost is nearly equal to the gain in the new component in the mixture albumin plus dextran. This appears to be true at all pH's between 9.6 and 6.6.

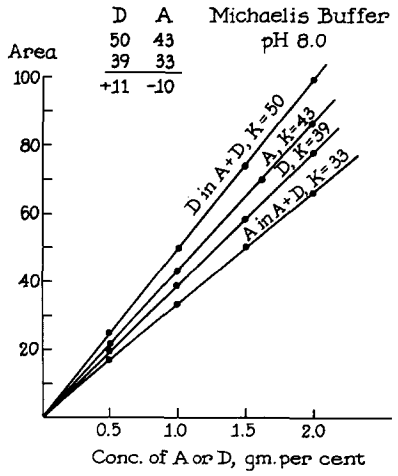

FIGURE 2. Ordinate, areas under spikes; abscissa, concentration of albumin or dextran. Line A for albumin, line D for dextran, line A in A + D for albumin in mixture, line D in A + D for dextran in mixture. The table inserted in the figure shows how the transfer of albumin to the dextran is calculated.

If the value for the refractive index increment of albumin is 0.0018, that for dextran is about 0.0015, and that for the new species in the mixture, albumin plus dextran, is about 0.00205. The new species seems to be composed of about one albumin molecule for every four dextran molecules, but the complex D_4A is only one of many possible complexes that would account for the observations. It is possible that only the high molecular portion of the dextran enters into the complex. This is a point, however, which would have to be investigated by using more polydisperse samples of dextran.

The foregoing is probably an oversimplified account of this interaction, which is neither instantaneous nor altogether stable. It has an attached time effect, for it is only about 80 per cent complete when the albumin and dextran have been mixed together for 5 minutes; further interaction takes place slowly up to 4 hours. The interaction also has a temperature coefficient not yet investigated.[3]

[3] Using the method of Horowitz *et al.* (1955) of injecting albumin and albumin plus dextran into the interface between xylene and buffer and measuring the interfacial tension corresponding to the

2. *Possible Interaction of Dextran and Gamma Globulin*

The 6 per cent dextran was dialyzed against Michaelis buffer at pH 6.4 and 8.6. Gamma globulin (Cohn's fraction II-1,2) was dissolved in Michaelis buffer at pH 6.4 and 8.6 in a concentration of 4 per cent; it was then dialyzed against the two buffers. The dextran and the gamma globulin solutions, at each of the two pH's, were then mixed in the proportions of 1.0 ml. dextran, 1.0 ml. buffer, and 2.0 ml. gamma globulin solution. After standing for 4 hours at 20°C., or even for shorter times, an attempt was made to obtain electrophoretic patterns.

This is a case in which the electrophoretic method is apparently unsuitable. One reason is that the electrophoretic velocity of gamma globulins is so small that the protein scarcely moves from the junction of the channels, while its mobility does not depend much on the pH above or below its isoelectric point (pH 7). Dextran behaves similarly, so we have two almost immobile components, which, even if they interact, do not give two spikes. A second reason is that 2 per cent gamma globulin is slightly opalescent in Michaelis buffer between pH 6.4 and 8.6; this makes it difficult to see the junction of the channels and the outline of the spike. In fact, the mixtures of dextran and gamma globulin show slight precipitation at either pH 6.4 or 8.6; some reaction or interaction apparently occurs, but electrophoresis does not seem to be suitable as a method for studying it.

3. *Absence of Interaction between Dextran and Fibrinogen*

Systems containing 2 per cent fibrinogen, 1.5 per cent dextran, and 2 per cent fibrinogen mixed with 1.5 per cent dextran were prepared at pH 6.6, 7.3, and 8.6. On electrophoresis, the first two systems give a fibrinogen and a dextran spike, the areas under each of which can be measured. Call them A_A and A_D. The system containing the mixture gives only one very slowly moving spike, and the area under it is $(A_A + A_D)$, to within $\pm$ 3 per cent, at all three pH's. The simplest interpretation of this is that there is no interaction between fibrinogen and dextran, or if there is, that the new species formed has virtually the same refractive index increment as fibrinogen and dextran have individually (0.0014 to 0.0015).

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addition of greater amounts of albumin or albumin plus dextran gives results which are definite but not helpful. At all pH's between 2.5 and 8.5, the interfacial tension decreases as the amount of albumin injected increases in exactly the same way as that observed when albumin plus dextran is injected. This means that the albumin spreads in the interface, whereas the dextran passes into the buffer; *i.e.*, the bonding which draws the albumin into the interface is much greater than the bonding between albumin and dextran.

It is also a pleasure to thank Dr. Walter H. Seegers and Dr. Kent D. Miller for giving us purified fibrinogen, Dr. Robert B. Pennell for supplying us with purified gamma globulin, and the American Red Cross for giving us fraction V (serum albumin).

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