Technical Papers

Interactions Between Proteins and Azosulfonamides[1]

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As part of a general study of the interactions of drugs with proteins, an investigation was undertaken of changes produced in the visible absorption spectra of some azosulfonamides in the presence of proteins, particularly bovine serum albumin. Large effects have been observed and may be attributed to the formation of a drug-protein complex.

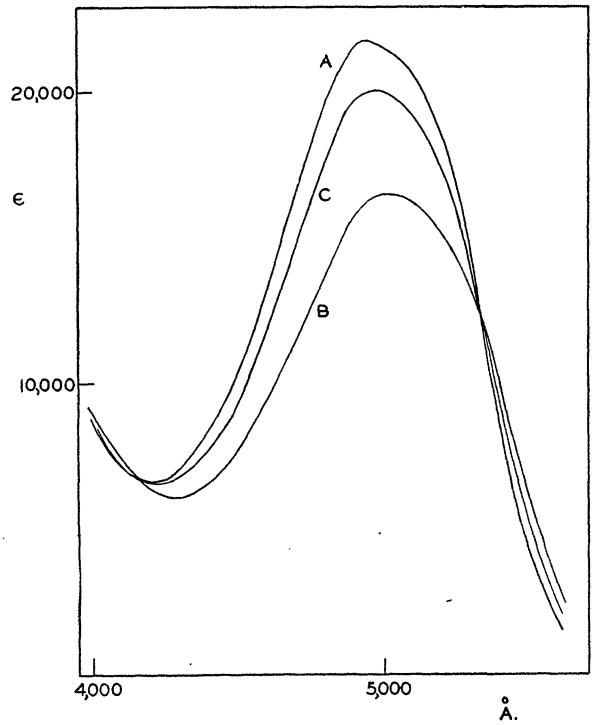

FIG. 1. Absorption spectra of azosulfathiazole in various aqueous media: A, buffer of pH 6.8; B, bovine serum albumin (2 grams/liter); C, $KHC_8H_4O_4$ and bovine serum albumin.

Curve A (Fig. 1) illustrates the absorption spectrum of one of the drugs, azosulfathiazole, in the presence of a phosphate buffer at a pH of 6.8. The same curve is obtained at all pH's from 2 to 9. Similarly, the spectrum is unaffected by the addition of sodium chloride, which alters the electrostatic field of the medium and has been known to change the absorption spectra of other colored anions (*1*).

On the other hand the addition of bovine serum albumin effects a pronounced change in the spectrum of azosulfathiazole, as can be seen in Curve B. Since pH and salt effects have been ruled out, the protein interaction must be due to specific complex-formation with the drug. That the phenomenon cannot be attributed merely to van der Waals' interaction in the presence of large molecules is illustrated by the fact that substances such as sodium dodecyl sulfate (with a molecular weight of 20,000 in the micellar state in aqueous solution, *3*) or Carbowax (a commercial polyoxymethylene polymer with a molecular weight of about 6,000, *2*) have no significant effect on the spectrum of azosulfathiazole.

A change in pH over a range of 4.8–9.2 shows no effect on the spectrum of the complex. Such behavior would indicate that the carboxyl groups of the protein are not involved in the complex-formation but that the quaternary nitrogens in the protein are the focuses of attachment.

That the drug-protein complex is quite strong has been corroborated by an approximate calculation of the first equilibrium constant from shifts in the spectra in solutions of variable protein concentration. These calculations lead to an estimate of 1×10^{-6} for K. Such an equilibrium constant corresponds to a free-energy change of about 8,000 calories in the binding process, an energy which indicates that the bond is somewhat stronger than that of a simple hydrogen-bond, probably because of an additional electrostatic attraction between a sulfonic acid anion of the drug and a quaternary nitrogen of the protein.

A very interesting reversal in the spectrum of the complex is observed upon the addition of potassium acid phthalate to the albumin-drug solution (Curve C). Apparently the complex between dye and protein has been largely, though not completely, disrupted. Such behavior would indicate a competition between the phthalate ion and drug ion for some group on the protein, probably a quaternary nitrogen, a competition which is strongly reminiscent of that between *p*-aminobenzoic acid and the simple sulfonamides.

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