From my experience, it is anticipated that the Weibull function will be useful in future work involving the quantitative interpretation of dissolution rate data.

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Urea increases serum albumin binding of drugs

Urea at concentrations higher than 2 M induces alterations in the molecular structure of bovine serum albumin (see for example Scheraga & Mandelkern, 1953; Gutter, Peterson & Sober, 1957; Williams & Foster, 1959; Santamaria, Fuerle & others, 1961).

We have investigated the effects of urea at physiological concentrations on the interactions of various drugs with serum proteins.

The drugs used doxycycline, metoclopramide, progesterone and sulfaethylthiazole, differ in chemical structure and physico-chemical features. For instance if the pH is increased from 5·2 to 9, the interaction of serum albumin with tetracyclines increases (Bononi, Pagnini & others, 1966) whereas the interaction with metoclopramide decreases (Pagnini & Di Carlo, 1972). Furthermore if the pH is increased from 5·5 to 11, the distribution coefficient chloroform/water decreases for tetracyclines (Von Wittenau & Yeary, 1963) whereas it increases for metoclopramide (Pagnini & Di Carlo, 1972).

Bovine serum albumin (Sigma Chem. Co. Frac. V) was purified from fatty acids (by extraction with acetic acid, 5% in isooctane, according to Goodman, 1957) and from ions (by dialysis in buffer tris HCl 0·05M, pH 7·4 after addition of EDTA).

The binding capability of the four drugs ($1{\cdot}13 \times 10^{-4}$M in buffer tris HCl 0·05M pH 7·4) with bovine serum albumin, with and without urea $1{\cdot}6 \times 10^{-3}$M, was assayed by the dialysis equilibrium technique (Klotz, Walker & Pivan, 1946). For this purpose 2 ml of bovine serum albumin solution ($5{\cdot}7 \times 10^{-4}$M) with or without urea was placed inside the dialysis tube (A. H. Thomas) and dialysed for 36 h at 5° against 6 ml of a solution of each drug. Then doxycycline (at 361 nm) and progesterone (at 240 nm) were assayed spectrophotometrically and metoclopramide and sulfaethylthiazole were assayed according to Bratton & Marshall (1939).

The binding capacity was expressed in terms of number of drug moles bound/mol of serum albumin (r).

The binding capability with serum albumin is greatest with sulfaethylthiazole >doxycycline >metoclopramide >progesterone (Fig. 1).

Addition of urea induces an increase of the binding capability of approximately 40% for all the drugs.

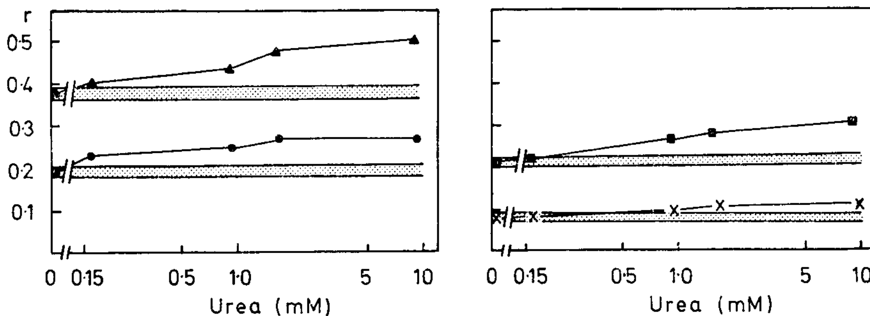

FIG. 1. Binding capability (r = mol of drug bound per mol of protein of doxycycline (■), metoclopramide (×), progesterone (●) and sulfaethylthiazole (▲) (all at the concentration of $1{\cdot}13 \times 10^{-4}$M) with bovine serum albumin ($5{\cdot}7 + 10^{-4}$M) with or without urea (at concentrations ranging from $1{\cdot}6 \times 10^{-4}$M and $9{\cdot}3 \times 10^{-3}$M).

The interaction of serum albumin with all four drugs in the presence of urea is concentration-dependent over a physiological concentration range of urea. Much higher concentrations can be found in pathological conditions and it could be that in such cases the increase of urea would affect the drug-protein binding.

However, although the concentrations used were much lower than those found to denature proteins, it seems logical to suggest that the urea-induced increase of the drug-protein binding may be related to a disorganization of the protein structures probably caused also by a reduction of the intramolecular hydrophobic forces (see for example Bruning & Holtzer, 1961; Coombes, Katchalski & Doty, 1960; Kauzmann, 1959; Waugh, 1954; Whitney & Tanford, 1962).

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