AN IN VITRO MODEL OF DRUG-ALBUMIN INTERACTION

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There are a number of in vitro filtration techniques to measure the binding of drugs to serum albumin; notably equilibrium dialysis, dynamic dialysis (Meyer & Guttman, 1968), ultrafiltration using visking tubing (Wishinsky et al, 1962) or millipore filters (Spector et al, 1972). All these methods presented problems in initial work and finally a modification of the ultrafiltration method of Blatt et al, 1968 was chosen to measure drug binding and to assess drug-drug interaction at albumin binding sites. The technique was modified so that instead of using a continuous washing procedure, serial additions of a drug stock solution were alternated with serial sampling of drug ultrafiltrate. The filtration cell was placed in a controlled temperature water bath during experimental runs.

Data on the binding of chlorpromazine, phenylbutazone and warfarin to a 0.2% solution of human serum albumin (A.B.Kabi, Stockholm) in phosphate buffer at pH 7.4 $\pm$ 0.1, were established and displacement of warfarin by phenylbutazone and vice-versa was assessed. A Fortran programme was written and used on-line to process these data.

Preliminary values of the numbers of first type binding sites (n) and their respective association constants (k) for these three drugs and for the warfarin-phenylbutazone combinations were determined (Table 1) from plots as drawn by Sellers & Koch-Weser (1970). The plots were analysed using a linear regression analysis programme on a Rockwell 920 calculator. A non-linear regression analysis computer programme, in initial use, gave decreased n values and increased k values. These values are not included in the table. All drugs appear to have two initial binding sites in the concentrations used (0-50µg/ml).

TABLE 1. Drug-serum albumin binding data.

DRUG	TEMP. ^{o}C	n	$k, 10^{4}\ M^{-1}$	nk
Warfarin sodium	37	1.93	9.04	17.44
Phenylbutazone	37	1.92	8.30	15.94
Chlorpromazine HCl	20	2.19	2.35	5.15
Combination (warfarin data)	37	2.37	1.92	4.55
Combination (phenylbutazone data)	37	1.86	5.64	10.49

(Data for each drug/albumin system are the average of four separate experimental runs).

Sellers & Koch-Weser (1970) have suggested that the product nk is a useful index of total binding affinity of protein for warfarin. The known total affinity of warfarin being decreased when in combination with phenylbutazone is well shown using the present experimental system. Also, the affinity of phenylbutazone in the presence of warfarin is decreased. In both instances, numbers of binding sites remain two (to nearest integer); these experimental data suggest mutual competitive interaction between these two drugs for common binding sites. This present model may prove suitable to follow the course of drug-drug interactions at this binding site.

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