albumin was four times greater than that using tromethamine buffer and 2% human serum albumin and about 20 times that determined for tromethamine and 1% human serum albumin. Furthermore, the number of sites in the first class (n_1) in phosphate buffer was substantially greater than in tromethamine. In the second class of sites, the association constant (k_2) and the number of sites (n_2) did not appear to show a strong dependence on the buffer used or the protein concentration. This suggests that tromethamine buffer did not interfere with binding in the second class and that binding to these sites possibly occurs by a less specific mechanism.

A comparison of the binding parameters obtained by Judis (1) and ourselves for the binding of tolbutamide to 2% human serum albumin in tromethamine buffer demonstrates the effect of neglecting the existence of the second class of sites. Although both sets of data are in good agreement on the Scatchard plot (Fig. 1), estimation of the primary association constant (k_1) from the slope of the linear portion of the plot does not correct for the slope contribution due to the second class of sites. This leads to an underestimate of the association constant on the order of 20% or 9000 l. mole^{-1} and a larger number of primary binding sites.

In the case of chlorpropamide, determination of binding in the presence of tromethamine buffer leads to a reduction in the number of binding sites and a fourfold reduction in the association constant relative to values obtained in phosphate buffer. Thus, as with tolbutamide, tromethamine strongly suppresses the binding.

These data illustrate the effects that buffers and other materials may have on the extent of protein binding *in vitro*. In such studies it is important to characterize the binding at several buffer or protein concentrations to test that the observed binding is a real effect rather than an artifact dependent on the particular buffer used. This also applies to salts which may be added to suppress the Donnan effect in equilibrium dialysis experiments. These considerations are also relevant to competitive binding studies. When the buffer system itself acts as a competitor and is present in high concentrations relative to the other competitors, the significance of the results is somewhat difficult to assess.

Judis (1) used two graphical methods (7, 8) to present his data, and there is excellent agreement between them. However, this is to be expected since the two methods are closely related. The plot used by Sandberg *et al.* (7) does not normalize the concentrations of bound small molecules for variations in total protein concentration and is for use chiefly when the protein concentration is not precisely known. The method used by Eichman *et al.* (8) is the well-known Scatchard plot, and in this case the results are normalized for variations in protein concentration. The only difference between the two is that, in the method of Sandberg *et al.*, the intercepts on the ordinate and abscissa are nkP_t and nP_t, respectively, where P_t is the concentration of protein. The value of n cannot be estimated without a knowledge of the protein concentration. In all other respects, both plots are essentially mathematically identical. Thus, any variation occurring in the value of n or k must only be the result of the errors expected in deriving information from graphical data.

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Binding of Sulfonylureas to Serum Albumin: A Response

Keyphrases ☐ Sulfonylureas—binding to serum albumin ☐ Protein binding—sulfonylureas to serum albumin ☐ Binding, protein—sulfonylureas to serum albumin ☐ Dialysis, equilibrium and dynamic—explanation of differences found studying sulfonylurea binding to serum albumin

Sir:

Crooks and Brown (1) suggested substantial disagreements between their findings and mine (2). Disagreement between the results is complicated by the fact that a different method was used in each article. Equilibrium dialysis is an old, established method for studying protein binding, and there is a substantial literature of findings with this experimental approach. The new method used by Crooks and Brown, dynamic dialysis, first described by Meyer and Guttman (3) has not been used as extensively as equilibrium dialysis.

Disagreement with the results (2) also may have occurred because Crooks and Brown did not repeat the experiments using equilibrium dialysis or the same methods for analysis, such as radiochemical assay. No data are offered (1) regarding the analytical work, interfering substances, sensitivity of the assays, or specificity of the analyses. Therefore, comparison of data may or may not be valid.

The data in their Fig. 1 (curve B) lead to the conclusion that using tromethamine buffer and 2% human serum albumin results in agreement with our findings.

The only variable was the dialysis method, that is, equilibrium dialysis (2) and dynamic dialysis (1). These findings are essentially an indication that the two dialytic methods may give equivalent results. Curve D, representing results using bovine serum albumin are not relevant since bovine serum albumin was not used in my work. Curve A, for 1% human serum albumin, suggests that binding characteristics change with protein concentration. Curve C, representing human serum albumin in phosphate buffer, suggests that protein concentration is of no consequence in phosphate buffer, although it is in tromethamine buffer. Figure 1 (1) thus leads to the conclusions that results with tromethamine buffer are different from those obtained with phosphate buffer and that protein concentration is of consequence in tromethamine buffer but not in phosphate buffer. These conclusions are not in disagreement with my findings because I made no claims about the generality of results in tromethamine buffer.

Figure 2 (1) is very difficult to interpret as presenting conflicting data because it represents data of experiments using 1% human serum albumin in phosphate and 1% bovine serum albumin in phosphate while only 2% human serum albumin was used in my work. In the experiments (2), the sulfonylureas usually were bound to the extent of 80% or higher at all concentrations of sulfonylurea used with the exception of the highest ($81–84 \times 10^{-6}$ mole) at which 40–60% binding was found, which is a rather substantial interaction.

A comment (1) was made that estimation of binding constants from extrapolation of the linear portion of Scatchard plots may be inaccurate. There is no disagreement on this point. The binding constants (2) were approximations because of this reason and, more basically, because of the limited number of data points. However, there is reasonable agreement in Table I between the n value for tolbutamide with 2% human serum albumin in tromethamine and that determined by Crooks and Brown. The difference between the n values for tolbutamide with 2% human serum albumin in tromethamine (2) and in *Reference 1* is less than that shown in Meyer and Guttman's paper (3) comparing k_1 for their kinetic method and literature values for ultrafiltration.

I cannot accept the conclusion of Crooks and Brown that there is questionable significance to my data on drugs competing with the binding of sulfonylureas to human serum albumin. Given that tromethamine may bind to human serum albumin, the amount of tromethamine was constant in each cell and the reduction in binding caused by the presence of competitor drugs was beyond that possibly caused by tromethamine. The reduction in binding caused by these competitor drugs was significant, and the results clearly have qualitative significance. Quantitative conclusions in terms of the amount of reduction in binding of sulfonylureas to human serum albumin caused by a number of moles of competitor drug would not be absolute if tromethamine also acts as a competitor drug; but with a constant amount of tromethamine in each system, the quantitative conclusions would be relative.

I believe Crooks and Brown (2) make two important contributions which do not represent disagreement with my findings (1) but actually extend our knowledge of binding of sulfonylureas to human serum albumin and, perhaps, protein binding in general. First, buffer systems may have significant effect on binding and more than one buffer system should be employed to ascertain these effects. Second, protein concentration may affect binding characteristics, although this finding is not new. Brunkhorts and Hess (4) found differences in binding parameters at different concentrations of albumin in studies of the interaction of the latter with cortisol. Perhaps this phenomenon, not yet satisfactorily explained, may be more general than is now appreciated.

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Importance of Considering Variables when Using Magnetic Basket Dissolution Apparatus

Keyphrases □ Magnetic basket dissolution apparatus—effect of basket mesh size and revolution speed □ Dissolution apparatus, magnetic basket—effect of basket mesh size and revolution speed

Sir:

In several recent articles (1, 2), the versatility and adaptability of the magnetic basket were demonstrated. This apparatus was initially developed to yield reproducible dissolution profiles for capsules. The adaptability of the magnetic basket apparatus to tablet dissolution was also discussed (2). By using several sets of specially formulated tablets as the control, the magnetic basket was shown to differentiate between the common tablet parameters of hardness, particle size, and formulation changes, thereby illustrating its possible use in quality control. However, to use the concept of the magnetic basket as a means of correlating *in vitro* dissolution with the *in vivo* performance of a drug, further adaptability of the basket must be considered. Since the above-mentioned tablet parameters are not appreciably changed and remain constant after initial clinical testing demonstrates the effectiveness of the dosage form, the dissolution system must be able to show further versatility in its ability to provide an *in vitro* dissolution profile which approximates the *in vivo* behavior of the drug. Adjustment of the *in vitro* conditions, such as propeller height and revolutions per minute, or a change in the mesh or size of the basket