positive slopes have been observed when the protein concentration has been varied (Bowmer & Lindup 1978). No satisfactory explanation for this phenomenon has as yet been put forward. However, care should be exercised in extrapolating from binding results obtained by varying the protein concentration to the situation in which the drug concentration is varied.

In conclusion the approach of Romer & Bickel, although it works for the examples chosen by them, should be viewed with caution for the several reasons listed above, which could invalidate it.

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Variation in the heats of reaction of drugs and albumin with the source and pretreatment of the albumin

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It has been shown previously that microcalorimetry can be used to investigate the interaction of drugs with macromolecules (Otagiri et al 1978) including those with human serum albumin (HSA). Primary binding constants, heats of reaction and entropy changes following the binding phenomena were reported. Recent investigations of the binding of salicylic acid to HSA have shown the heat of reaction but not the free energy, for the binding of the first salicylate molecule, to be greatly dependent upon the source and pretreatment of the albumin (Table 1). The measurements were made in an LKB flow microcalorimeter using the serial dilution technique (Hardee et al 1978) and using an iterative least squares technique to calculate the first binding constant and the heat of reaction at the first binding site.

Table 1. Derived parameters for salicylic acid albumin interaction.

HSA Source	ΔG (J mol^{-1})	ΔH (J mol^{-1})*	ΔS (J mol^{-1} deg^{-1})	n**
Fraction V	−30 000	−35 000 ± 400	−18	15
Deionized Fraction Five	−32 000	−37 000 ± 400	−17	15
Fraction V + 0·075 M NaCl	−33 000	−24 000 ± 600	+30	14
Crystalline	−30 000	−24 000 ± 500	+20	13
Deionized crystalline	−25 000	−40 000 ± 600	−50	20
Deionized crystalline + 0·075 M NaCl	−28 000	−22 000 ± 300	+18	16
Fatty acid free	−36 000	−40 000 ± 500	−14	14
Plasma	−21 000	−55 000 ± 2600	−112	13
Bovine serum albumin	−32 000	−37 000 ± 1000	−17	15

All measurements are made at 25·0 °C, in 0·1 M phosphate buffers of pH 7·40 using the LKB Model 2107–121.
*Standard error estimate from asymptotic correlation $\alpha = 0{\cdot}05$.
**n = number of data points.

The three albumins were from normal commercial sources and gave similar optical densities at 210 and 278 nm (Perrin & Vallner 1975) and at 615 nm following the interaction with bromocresol green (Rodkey 1965). The albumins also gave similar intrinsic optical activities (Perrin & Vallner 1975). L. H. M. Jansen & T. H. A. Nelen (submitted for publication) have recently shown that sulphaethidole displaces chloride ion from bovine serum albumin and unpublished observations in the same laboratory have shown that warfarin displaces chloride ion from HSA.

An explanation of the data in Table 1 is that the albumins contain a small amount of inorganic ions, possibly chloride, and the low affinity of these ions for HSA compared to the salicylate results in little change in the derived binding constant but the displacement of the chloride by the drug results in a large effect on the heat of reaction. The deionization was carried out using Amberlite IR120 and IRA400 as recommended by Jansen & Nelen and seems to be an essential pretreatment of HSA before use in any binding studies, particularly if heats of reactions are to be measured. Gel electrophoresis of the three HSA samples showed that all had the same bands present, however the fraction of the total albumin in the minor bands increased as the number of purification steps was increased. The defatted material seems to be far less pure than the fraction V. Defatting would appear to be an unnecessary complication in binding studies, particularly after the deionization treatment. The binding constant in plasma was significantly lower than in the albumin solutions, but the heat evolution was higher. This may be due to competition between the drug and plasma components for the binding site on albumin as well as the binding of the salicylate to other proteins in the plasma. Although the data presented represent the interaction of salicylate with HSA, similar results have been obtained for the interaction of sulphaethidole with HSA.

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