Binding of Sulfonylureas to Serum Proteins

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Abstract □ Three sulfonylureas, acetohexamide, chlorpropamide, and tolbutamide, were investigated in terms of their affinity for the major human serum proteins. Equilibrium dialysis was used to determine protein binding with radioactive derivatives. In tromethamine buffers at pH 6.5, 7.4, and 8.4 and serum protein concentrations approximately equal to that in normal human serum, the sulfonylureas were bound, to the greatest extent, to albumin (65–88% with acetohexamide, 57–72% with chlorpropamide, and 80–86% with tolbutamide). No significant binding occurred with the other serum proteins to acetohexamide except fibrinogen I (10%) and α-globulin IV-4 (7–16%). There was some binding of acetohexamide to β-lipoprotein III-0 (up to 4.2%). No significant binding of chlorpropamide could be demonstrated to any other serum proteins except β-globulin III (13.5%) and α-globulin IV-4 (4%). Tolbutamide was bound to β-globulin III (15.8%) and α-globulin IV-4 (12.9%). With albumin, *n* values at pH 6.5, 7.4, and 8.4 were: 1.39, 1.37, and 1.13, respectively, for acetohexamide; 1.93, 1.67, and 1.43, respectively, for chlorpropamide; and 1.78, 1.34, and 1.26, respectively, for tolbutamide. Inhibition of protein (albumin) binding of the sulfonylureas by drugs known to potentiate their action was studied. Substantial inhibition of binding of chlorpropamide was found with molar concentrations of 1–20 times that of the sulfonylurea of sodium salicylate, aspirin, phenylbutazone, sulfadimethoxine, sulfaphenazole, and sulfisoxazole. In the case of acetohexamide, substantial inhibition of protein binding was found with sulfaphenazole and phenylbutazone, and lesser but significant similar effects were found with sodium salicylate, aspirin, sulfadimethoxine, and sulfisoxazole. Binding of tolbutamide was inhibited by sulfaphenazole and phenylbutazone most markedly and somewhat less by sodium salicylate, aspirin, sulfadimethoxine, and sulfisoxazole.

Keyphrases □ Protein binding, human serum—radiolabeled sulfonylureas, relationship between inhibition and hypoglycemia potentiation □ Sulfonylureas—binding to human serum proteins, relationship between inhibition and hypoglycemia potentiation □ Drug-protein binding—sulfonylureas

Although the occurrence of severe and protracted hypoglycemia has been relatively rare in the more than 10 years that sulfonylureas have been used in the treatment of diabetic patients (1), there have been reports of such patients who experienced severe hypoglycemia. Most of the reported cases involved the administration of chlorpropamide (1, 2), but several were related to tolbutamide (1). In addition, some reports in the literature indicated that other medicinal agents cause significant potentiation of the action of sulfonylureas (1, 3–6). Various mechanisms were proposed including inhibition of metabolism of the sulfonylurea by the liver (7, 8), interference with the excretion of a metabolite of a sulfonylurea (9), increasing the half-life of the sulfonylurea (8), and displacement of sulfonylureas from the blood proteins to which they are bound (2, 5, 9–11). Substantial evidence for the first three theories has not been forthcoming, but some experimental evidence was presented for the latter theory (4, 5, 11).

It has been well established that the sulfonylureas are strongly bound to serum proteins (4, 5, 11–14). The conclusions of these studies included: (*a*) sulfonylurea compounds are strongly bound to serum proteins, (*b*) some specificity exists in terms of which serum protein a specific sulfonylurea is bound to, and (*c*) certain drugs interfere with binding of a sulfonylurea to serum proteins. Wishinsky *et al.* (11) showed that four sulfonylureas (chlorpropamide, carbutamide, metahexamide, and tolbutamide) were bound to bovine albumin rather strongly at a drug concentration of 5 mg./100 ml.; the percentage of binding at this concentration ranged from 82 to 100%. On the basis of electrophoretic experiments, metahexamide was shown to be bound primarily to α-globulin while tolbutamide was bound primarily to albumin and prealbumin. If salicylate was added to the drug–bovine albumin system, a decreased percentage of binding of sulfonylurea resulted. Christensen *et al.* (5) found that the addition of a number of sulfonamides and phenylbutazone decreased the binding of tolbutamide to whole human serum. However, all of these reported findings must be considered preliminary because:

1. Binding of sulfonylureas to serum proteins was not treated in the quantitative fashion typical of proper studies of protein binding (15).

2. Quantitative determination of the sulfonylurea compounds was chemical, and this approach is not always as specific or sensitive in physiological concentrations as with the utilization of these compounds labeled with radioactive atoms.

3. A truly complete study of the ability of those drugs able to potentiate the action of sulfonylurea compounds

Table I—Characteristics of Binding of Sulfonylureas to Human Serum Albumin[a]

Sulfonylurea	pH of Reaction Mixture	—Determined by Method I[b]— *n*	*k*	—Determined by Method II[c]— *n*	*k*
Acetohexamide	6.5	1.419	35,951	1.39	36,230
	7.4	1.385	33,948	1.37	34,611
	8.4	1.153	53,144	1.13	55,043
Chlorpropamide	6.5	1.968	10,127	1.93	10,441
	7.4	1.641	10,873	1.67	10,589
	8.4	1.412	10,296	1.43	10,147
Tolbutamide	6.5	1.77	25,714	1.78	25,517
	7.4	1.36	40,555	1.34	40,651
	8.4	1.26	39,796	1.26	39,762

[a] Each cell contained 20 mg. of albumin. [b] A plot of D_b/D_f *versus* D_b. See *Methods and Materials*. [c] A plot of r/A *versus* r. See *Methods and Materials*.

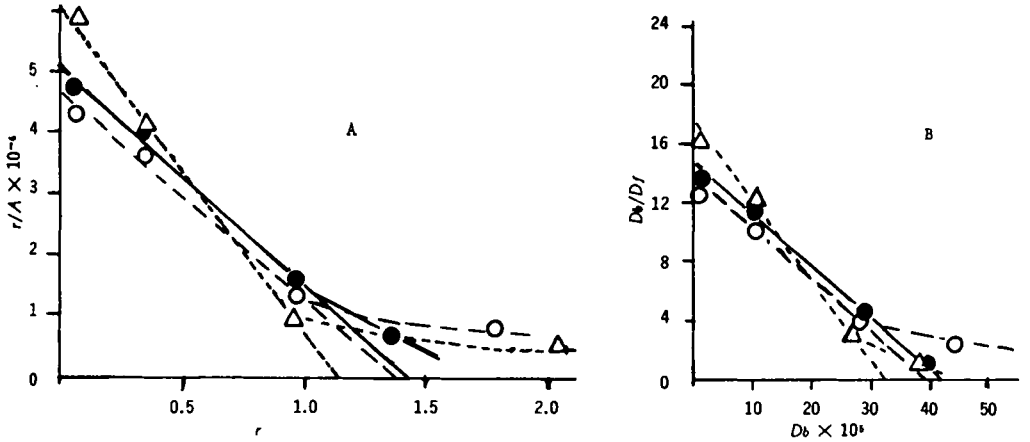

Figure 1—*Binding of acetohexamide to human serum albumin. Each dialysis system contained 2.9 × 10^{-7} mole of albumin and one o the following concentrations of acetohexamide: 2, 11.88, 41.5, or 81 × 10^{-8} mole. A = plot of* r/A versus r. *B = plot of* D_b/D_f versus D_b. *Key: ●, pH 6.5, correlation coefficients (Pearson's* r*) for Plot A = 0.9968 and for Plot B = 0.9969; ○, pH 7.4, correlation coefficients (Pearson's* r*) for Plot A = 0.9965 and for Plot B = 0.9979; and △, pH 8.4, correlation coefficients (Pearson's* r*) for Plot A = 0.9998 and for Plot B = 1.000.*

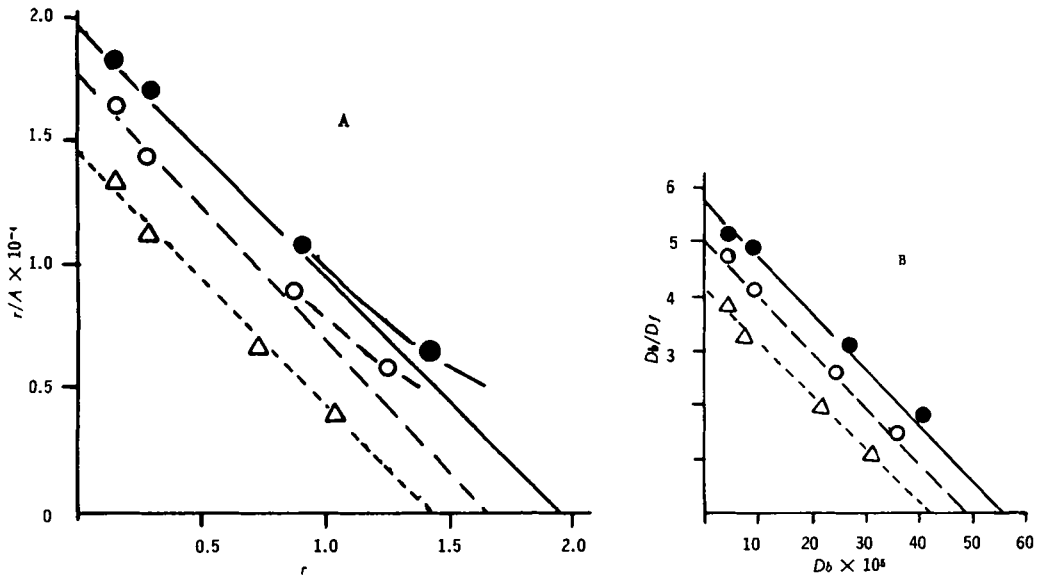

Figure 2—*Binding of chlorpropamide to human serum albumin. Each dialysis system contained 2.9 × 10^{-7} mole of albumin and one of the following concentrations of chlorpropamide: 6, 12, 42, or 84 × 10^{-8} mole. A = plot of* r/A versus r. *B = plot of* D_b/D_f versus D_b. *Key: ●, pH 6.5, correlation coefficients (Pearson's* r*) for Plot A = 0.9982 and for Plot B = 0.9927; ○, pH 7.4, correlation coefficients (Pearson's* r*) for Plot A = 0.9974 and for Plot B = 1.000; and △, pH 8.4, correlation coefficients (Pearson's* r*) for Plot A = 0.9867 and for Plot B = 0.9917.*

Table II—Binding of Acetohexamide to Various Human Serum Proteins[a]

Protein	Final Concentration of Protein, mg./ml.	—Percent Binding at pH— 6.5	7.4	8.4
Fibrinogen I	1.0	0	0.6	0.9
	2.0	0.2	1.1	11.1
γ-Globulin II	3.0	0	0	0
	6.0	0	0	0
β-Globulin III	3.5	0.1	1.86	0
	7.0	—	—	0.66
β-Lipoprotein III-0	1.5	1.7	2.5	0
	3.0	3.3	4.2	0.6
α-Globulin IV-1	0.5	1.0	0.9	0.7
	1.0	0.4	1.1	0.9
α-Globulin IV-4	0.5	10.2	7.4	12.6
	1.0	12.2	16.4	11.0
Albumin	20.0	85.1	88.1	85.0

[a] Each reaction mixture contained 2 × 10^{-8} mole of acetohexamide.

to interfere with the binding of the latter to serum proteins has not been reported.

It is important that the concentration of potentiating drugs be chosen to bear some realistic molar concentration relative to the concentration of sulfonylurea and not simply some convenient multiple of an arbitrarily chosen concentration such as 5 or 10 mg./100 ml.

Thus, because displacement of protein bound sulfonylurea appeared to be the most likely explanation of this potentiation or drug interaction, we felt that it should be investigated in terms of the three major sulfonylureas in current clinical use and selected potentiating drugs. Radiotracer-labeled sulfonylureas were commercially available and thus made possible the experimental determination of effects on protein binding by potentiating drugs with precision, sensitivity, and specificity. In addition to these studies, it seemed valuable to investigate important characteristics of binding of

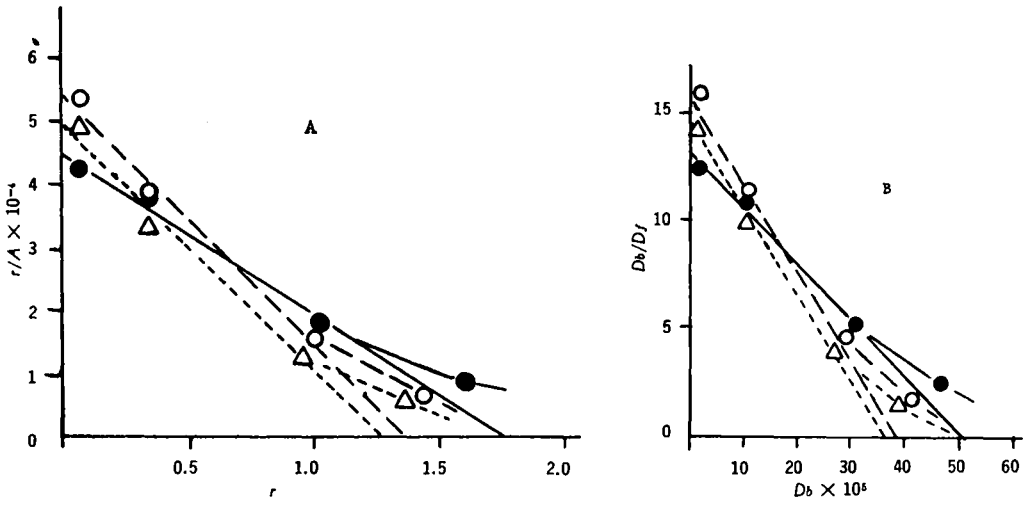

Figure 3 --*Binding of tolbutamide to human serum albumin. Each dialysis system contained 2.9 × 10^{-7} mole of albumin and one of the following concentrations of tolbutamide: 2, 12, 42, or 84 × 10^{-8} mole. A = plot of* r/A versus r. *B = plot of* D_b/D_f versus D_b. *Key:* ●, *pH 6.5, correlation coefficients (Pearson's* r) *for Plot A = 0.9962 and for Plot B = 0.9942;* ○, *pH 7.4, correlation coefficients (Pearson's* r) *for Plot A = 0.9961 and for Plot B = 1.00; and* △, *pH 8.4, correlation coefficients (Pearson's* r) *for Plot A = 0.9932 and for Plot B = 1.00.*

sulfonylureas to human serum albumin and to determine the specificity of binding to various serum proteins.

MATERIALS AND METHODS

Materials—Human serum proteins were obtained from a commercial source[1], and human serum albumin was purchased as the crystalline grade; all other serum fractions were the purified fractions bearing the identity indicated. The sulfonamides used were of USP or reagent grade. Sulfaphenazole[2], acetohexamide[3] both in the unlabeled and ^{14}C-labeled forms, unlabeled samples of chlorpropamide[4], and unlabeled samples of tolbutamide[5] were received as gifts. Salicylates were of USP or reagent grade, and chlorpropamide-^{35}S and tolbutamide-^{35}S were obtained from a commercial source[6]. All other chemicals were reagent grade.

Methods—Equilibrium dialysis was effected in Plexiglas multicavity dialysis cells of 1-ml. capacity in each of the two sides in a cell separated by a cellophane membrane[7]. Acetohexamide-^{14}C had a specific activity of 12.4 μc./mg. and was made up in a stock solution with a concentration of 0.26 mg./ml. of *M*/15 tromethamine buffer, pH 7.4. Chlorpropamide-^{35}S, specific activity of 1.6 mc./mmole, was dissolved in pH 6.5 tromethamine buffer in a concentration of either 0.66 or 1.32 mg./ml. The sample of tolbutamide-^{35}S used had a specific activity of 5.5 mc./mmole, and the stock solution of this drug was made with pH 8.4 tromethamine buffer as solvent; the concentration was either 0.22 or 1.32 mg./ml.

Proteins or protein fractions were dissolved in the buffer of the pH used in the dialysis unless otherwise noted. In a typical dialysis setup, 0.5 ml. of protein solution and 0.5 ml. of tromethamine buffer of appropriate pH were placed on one side of the membrane. The appropriately labeled sulfonylurea was placed on the other side of the membrane together with unlabeled sulfonylurea where necessary to provide the proper total concentration of the latter, an inhibitor in those experiments in which competitors for binding of sulfonylureas to proteins were studied, and enough tromethamine buffer to make a total volume of 1 ml. The dialysis cells were immersed in a 40° water bath and attached to a Burrell wrist-action shaker. Samples of 25 μl. were taken from each side of the membrane at time intervals of 0, 17, and 24 hr. of incubation. In all cases, equilibrium had been reached by 17 hr., and the results at 24 hr. were identical with the results at 17 hr. Radioactivity was determined in a liquid scintillation system[8], and all counts were taken to the 5% two sigma error. A dioxane–naphthalene–2,5-diphenyloxazole–dimethyl-[1,4-bis-2-(5-phenyloxazolyl)-benzene] scintillation solution was used throughout (16).

In the analysis of binding characteristics of the three sulfonylureas, the data were plotted both by the method described by Eichman *et al.* (17) and that described by Sandberg *et al.* (18). The slopes and intercepts were calculated by means of the standard formula for "least squares" (19, 20). In the methods of Sandberg *et al.* (18) and of Rosenthal (21), a plot is made of D_b/D_f as a function of D_b. The ordinate intercept, abscissa intercept, and slope are estimates of nkP_t, nP_t, and k, respectively; D_b is the molar concentration of bound drug, D_f is the molar concentration of unbound drug, n refers to the number of binding sites, k is the association constant for the binding of small molecules by sites on the protein, and P_t refers to total molar protein concentration. The method for plotting protein binding data cited by Eichman *et al.* (17) involves a plot of $r/(A)$ *versus* r. This results in a straight line of slope, $-k$, and ordinate and abscissa intercepts of nk and n, respectively. In this method, r equals moles of small molecule bound per mole of protein, n equals the number of binding sites available on each protein molecule, k equals the association constant for the reaction, and (A) equals the concentration of unbound small molecule.

RESULTS AND DISCUSSION

A limited number of studies have been reported on binding of sulfonylureas to serum proteins. The methodology used in several examples involved primarily such techniques as ultracentrifugation (22), equilibrium dialysis (5, 11, 15), ultrafiltration (11), and electrophoresis (11, 14). In the study reported here, equilibrium dialysis was chosen as the method for studying protein binding of the major sulfonylureas because it lent itself best to the determination of: (*a*) protein binding at a number of concentrations of the organic molecule, (*b*) the effect of various other organic molecules in a variety of concentrations on protein binding of sulfonylureas, and (*c*) binding of sulfonylureas by a variety of serum proteins or protein fractions.

Human serum albumin has been shown to bind a rather extensive variety of organic molecules (15, 23, 24); in those studies defining binding of various molecules by serum albumin, two characteristic constants have been calculated. One is the n value or the number of binding sites on the albumin molecule. The other constant is denoted by a variety of symbols, often simply k, and is the intrinsic association constant for the binding of a small molecule by sites on the protein molecule. These two characteristic constants were calculated

[1] Nutritional Biochemicals Corp., Cleveland, Ohio.
[2] Purdue-Frederick Co.
[3] Eli Lilly and Co.
[4] Chas. Pfizer Co.
[5] The Upjohn Co.
[6] Amersham/Searle Corp.
[7] Chemical Rubber Co. multicavity dialysis cells, 1 ml., No. 9355/412.
[8] Beckman model LS-133.

Table III—Binding of Chlorpropamide to Various Human Serum Proteins[a]

Protein	Final Concentration of Protein, mg./ml.	Percent Binding at pH 6.5	Percent Binding at pH 7.4	Percent Binding at pH 8.4
Fibrinogen I	1.0	0.1	0.3	0.8
	2.0	1.1	0	0
γ-Globulin II	3.0	0.1	0.8	1.0
	6.0	1.7	0	0
β-Globulin III	3.5	0.5	13.5	0
	7.0	—	—	2.8
β-Lipoprotein III-0	1.5	1.2	0.8	0.5
	3.0	1.4	0	0
α-Globulin IV-1	0.5	0.5	0	1.2
	1.0	0.3	0.4	0
α-Globulin IV-4	0.5	2.9	2.3	4.1
	1.0	13.0	2.4	3.6
Albumin	20.0	68.0	72.4	57.5

[a] Each reaction mixture contained 12×10^{-8} mole of chlorpropamide.

Table IV—Binding of Tolbutamide to Various Human Serum Proteins[a]

Protein	Final Concentration of Protein, mg./ml.	Percent of Binding at pH 6.5	Percent of Binding at pH 7.4	Percent of Binding at pH 8.4
Fibrinogen I	1.0	0.6	0	0
	2.0	1.0	0	0
γ-Globulin II	3.0	0.5	0	0
	6.0	0	0	0
β-Globulin III	3.5	1.5	15.8	0
	7.0			0
β-Lipoprotein III-0	1.5	0	0.1	0
	3.0	0	0.8	0
α-Globulin IV-1	0.5	0.3	0.7	0.5
	1.0	0	1.7	0
α-Globulin IV-4	0.5	12.9	4.2	7.3
	1.0	8.4	7.5	3.5
Albumin	20.0	81.6	86.6	80.2

[a] Each reaction mixture contained 12×10^{-8} mole of tolbutamide.

for the three sulfonylureas treated in this study at three pH values, and the results are listed in Table I. The binding of each of the sulfonylureas at four different concentrations and three pH values were calculated and plotted by two different methods (Figs. 1–3). Correlation coefficients were calculated (20) for all experimental points falling about the straight-line portions of the curves (Figs. 1–3) obtained by least-squares analysis, and the correlation coefficients indicated correlations significant to at least 0.1 in all cases and 0.05 or better in almost all of the plots ($n - 2$ degrees of freedom). The results obtained were rather similar; as the pH was increased, the n value decreased for all three sulfonylureas. The k values for chlorpropamide were approximately the same at all pH's used, but in the case of the other two sulfonylureas, the k values were higher at the higher pH. Since the affinity of a small molecule for a protein is a summation of a number of factors, it is rather difficult to interpret the effect of pH on k. For example, a change in pH may affect ionization of the small molecule and/or the protein as well as influence the number of binding sites exposed and available for binding. The lowest k values were obtained with chlorpropamide and the highest with acetohexamide, and this finding would suggest that acetohexamide is more strongly bound than either of the other two sulfonylureas while chlorpropamide is least strongly bound. This is in partial disagreement with the conclusions of Wishinsky *et al.* (11) who concluded, on the basis of percent binding, that binding tendency was in the increasing order of metahexamide, chlorpropamide, and tolbutamide. However, percent binding is not as accurate an analytical approach to determination of binding tendency as the calculation of k values.

Wishinsky *et al.* (11) also reported on electrophoretic determination of binding of sulfonylureas to whole serum. They found that metahexamide, a compound structurally related to acetohexamide, is bound to α-globulin at concentrations below 11.6 mg.%; at or above this concentration, the additional or excess drug was bound to albumin. They also showed that tolbutamide was bound primarily to albumin and also to prealbumin. Johnson *et al.* (14) demonstrated by electrophoresis that chlorpropamide was bound primarily to serum albumin. In an attempt to repeat these electrophoretic studies to confirm that the sulfonylureas were bound primarily to serum albumin and also to determine whether there is significant binding to other serum proteins, it was found that the electrophoretic method utilizing barbital buffer at pH 8.6 was unsatisfactory because all three sulfonylureas migrated at about the same rate as serum albumin; therefore, it was not possible to conclude whether binding to the latter protein did occur. This type of migration of the sulfonylureas is to be expected because their pKa values all lie between 5.3 and 6.5 (25–27), only slightly higher than that of albumin (24). Thus, the experimental approach used to determine protein binding was equilibrium dialysis with the various purified human serum proteins commercially available. The results obtained are given in Tables II–IV. The various serum proteins were reconstituted to concentrations approximating their concentrations in whole human serum (28, 29), and it can be seen that the highest percent binding at the three different pH's used was to albumin. At pH 6.5, a substantial amount of binding of chlorpropamide could be shown to α-globulin IV-4. This same protein bound a significant amount of acetohexamide at all three pH values and also bound tolbutamide most strongly at pH 6.5. None of the other serum proteins bound the sulfonylureas with the exception of chlorpropamide to β-globulin III at pH 7.4. Therefore, albumin is the protein to which the major binding of sulfonylureas occurs.

In view of the numerous clinical reports of interaction of various medicinal agents with sulfonylureas resulting in potentiation of the hypoglycemic action of the sulfonylureas, it appeared important to test the theory that this potentiation is the result of displacement of sulfonylureas from serum proteins. Competition between sulfonylureas and other compounds for binding sites on serum albumin was demonstrated by a number of investigators (4, 5, 11, 22). The majority of reports on potentiation interactions with sulfonylureas involve sulfonamides, salicylates, and phenylbutazone; in the studies described in this paper, representatives of these groups were chosen. The concentrations of the competitor were adjusted to be equal to that of the sulfonylurea on a molar basis and some multiple of the latter. The results given in Tables V–VII indicate percent binding in the presence of various concentrations of competitor and also the r values (moles of small molecule bound per mole of protein). From

Table V—Inhibition of Albumin Binding of Acetohexamide[a] by Various Medicinal Agents

Competitor	Concentration of Competitor ($\times 10^{-8}$ mole): 0 Percent Binding	0 r	12 Percent Binding	12 r	23.76 Percent Binding	23.76 r	47.52 Percent Binding	47.52 r	237.6 Percent Binding	237.6 r
Sulfaphenazole	84.2	0.345	78.2	0.3202	67.4	0.2759	53.5	0.2193	19.5	0.0797
Sodium salicylate	89	0.3520	82.4	0.3370	79.7	0.3260	75.7	0.3100	52.2	0.2138
Aspirin	86.4	0.3530	84.9	0.3476	83.9	0.3438	81.7	0.3348	58.9	0.2417
Phenylbutazone	86.1	0.3490	84.5	0.3466	79.8	0.3255	69	0.2828	38.6	0.1583
Sulfisoxazole	84.2	0.3452	83.6	0.3428	77.5	0.3172	74	0.3038	46.4	0.1900
Sulfadimethoxine[b]	86.8	0.3555	81.0	0.3317	74.7	0.3055	63.2	0.2593	46.8	0.1917

[a] The amount of acetohexamide per reaction mixture was 12×10^{-8} mole, and 2.9×10^{-7} mole of albumin was present. The pH was 7.4 throughout. [b] The amounts of sulfadimethoxine were, respectively: 0, 12×10^{-8} mole, 23.76×10^{-8} mole, 35.64×10^{-8} mole, and 71.28×10^{-8} mole.

Table VI—Inhibition of Albumin Binding of Chlorpropamide[a] by Various Medicinal Agents

Competitor	Concentration of Competitor (× 10^{-8} mole) 0 Percent Binding	0 r	12 Percent Binding	12 r	23.76 Percent Binding	23.76 r	47.52 Percent Binding	47.52 r	237.6 Percent Binding	237.6 r
Sulfaphenazole	65.5	0.2710	60.3	0.2497	53.2	0.2203	40.3	0.1669	13.1	0.0541
Sodium salicylate	68.5	0.2834	64.7	0.2676	57.8	0.2393	54.4	0.2252	29.9	0.1238
Aspirin	73.0	0.3021	71.8	0.2972	68.6	0.2838	55.9	0.2134	36.1	0.1493
Phenylbutazone	70.7	0.2928	60.6	0.2486	49.2	0.2038	38.9	0.1610	15.5	0.0641
Sulfisoxazole	65.9	0.2728	63.2	0.2614	59.0	0.2446	52.8	0.2183	27.7	0.1148
Sulfadimethoxine[b]	67.4	0.2790	64.4	0.2662	58.8	0.2431	40.7	0.1683	27.4	0.1131

[a] The amount of chlorpropamide per reaction mixture was 12 × 10^{-8} mole, and 2.9 × 10^{-7} mole of albumin was present. The pH was 7.4 throughout. [b] The amounts of sulfadimethoxine in the last two columns were: 59.4 × 10^{-8} mole and 115.8 × 10^{-8} mole, respectively.

Table VII—Inhibition of Albumin Binding of Tolbutamide[a] by Various Medicinal Agents

Competitor	Concentration of Competitor (× 10^{-8} mole) 0 Percent Binding	0 r	12 Percent Binding	12 r	23.76 Percent Binding	23.76 r	47.52 Percent Binding	47.52 r	237.6 Percent Binding	237.6 r
Sulfaphenazole	85.1	0.3522	81.0	0.3352	73.9	0.3058	59.6	0.2468	21.5	0.0889
Sodium salicylate	84.0	0.3478	84.1	0.3480	79.7	0.3300	72.5	0.3001	49.4	0.2042
Aspirin	83.5	0.3453	82.9	0.3431	79.8	0.3303	76.4	0.3161	54.1	0.2238
Phenylbutazone	87.2	0.3607	81.8	0.3385	65.6	0.2715	51.2	0.2119	28.0	0.1160
Sulfisoxazole	84.2	0.3486	83.6	0.3459	81.7	0.3382	75.0	0.3102	48.8	0.2020
Sulfadimethoxine[b]	85.9	0.3553	80.7	0.3339	73.4	0.3035	56.7	0.2348	41.3	0.1708

[a] The amount of tolbutamide per reaction mixture was 12 × 10^{-8} mole, and 2.9 × 10^{-7} mole of albumin was present. The reaction mixture had a final pH of 7.4. [b] The amounts of sulfadimethoxine in the last two columns were: 59.4 × 10^{-8} mole and 115.8 × 10^{-8} mole, respectively.

the reduction in percent binding with increasing concentration of competitor, it appears that sulfaphenazole is the most potent inhibitor of binding of sulfonylureas to serum albumin while aspirin is the least potent. Of the three sulfonylureas studied, the greatest decrease in percent binding by all of the competitors examined was caused with chlorpropamide. Although these studies of competitors of binding of sulfonylureas by human serum albumin do not exclude other possible mechanisms by which the hypoglycemia effects of sulfonylureas are potentiated, the results are clearly consistent with the theory that the potentiating medicinal agents displace sulfonylureas from serum albumin.

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