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THE DISPLACEMENT OF PHENYLBUTAZONE-^{14}C AND WARFARIN-^{14}C FROM HUMAN ALBUMIN BY VARIOUS DRUGS AND FATTY ACIDS*

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Abstract—The technique of ultrafiltration was employed to characterize the effect of various drugs and fatty acids on the binding of phenylbutazone-^{14}C and warfarin-^{14}C to human albumin. Tolbutamide, indomethacin, sulfamethoxypyridazine, and chlorophenoxyisobutyric acid (CPIB) competitively inhibit the binding of phenylbutazone-^{14}C to albumin. Similarly, CPIB at a concentration of $4{\cdot}6 \times 10^{-4}$M competitively inhibits the binding of warfarin-^{14}C to albumin. Phenylbutazone-^{14}C and warfarin-^{14}C appear to bind to the same site on the protein, since each drug competitively inhibits the binding of the other to human albumin.

Lauric acid, myristic acid, and stearic acid displace both phenylbutazone-^{14}C and warfarin-^{14}C from albumin. Lauric acid inhibits the binding of phenylbutazone-^{14}C and warfarin-^{14}C by competing with these compounds for the same binding site on the protein.

THE EXTENT of binding of acidic drugs to albumin may be influenced by the presence of other drugs which also bind to the protein.[1–3] Several studies have demonstrated that the pharmacologic effect of a highly bound drug may be increased when the drug is displaced from its binding site by another drug.[4, 5]

The present study characterizes the effect of various drugs and fatty acids on the binding of phenylbutazone-^{14}C and warfarin-^{14}C to human albumin.

METHODS

Human albumin (chromatographically isolated, 96 per cent pure), stearic acid, lauric acid, and myristic acid were obtained from the Mann Research Laboratories. Other drugs were obtained as follows: chlorophenoxyisobutyric acid (CPIB) from Ayerst Laboratories, phenyl-butazone from Geigy Pharmaceuticals, tolbutamide from the Upjohn Company, indomethacin from Merck & Co., and sulfamethoxypyridzine from Lederle Laboratories. Dr. Collin H. Schroeder of the Wisconsin Alumni Research Foundation generously provided samples of warfarin and warfarin-^{14}C. The radioactive compound was labeled in the 4-position of the coumarin moiety and had a sp. act. of 5·9 mc/m-mole. Phenylbutazone (phenyl-U-^{14}C) with a sp. act.

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of 1 mc/m-mole was provided through the courtesy of Drs. Rolf Denss and Murray Weiner of Geigy Pharmaceuticals.

Human albumin, tolbutamide, warfarin, and warfarin-^{14}C were dissolved in a potassium dihydrogen phosphate–disodium hydrogen phosphate buffer, ionic strength 0·1 and pH 7·4. Phenylbutazone, phenylbutazone-^{14}C, sulfamethoxypyridazine, CPIB, and indomethacin were dissolved in 0·1 N NaOH. Stearic acid, lauric acid, and myristic acid were dissolved in absolute ethyl alcohol.

The ultrafiltration technique of Rehberg was used to determine the extent of binding of warfarin-^{14}C and phenylbutazone-^{14}C to albumin.[6] Dialysis bags were prepared as described previously.[3] Each bag contained 0·5 ml albumin solution, 0·1 ml warfarin-^{14}C (0·1 μc) or 0·1 ml phenylbutazone-^{14}C (0·1 μc), 0·1 ml of drug solution, and 4·3 ml of the buffer. The final concentration of albumin in all studies, unless otherwise stated, was $1{\cdot}0 \times 10^{-4}$M. The extent of binding of phenylbutazone-^{14}C to albumin was measured over a range of concentrations of drug extending from $3{\cdot}4 \times 10^{-5}$ to $9{\cdot}0 \times 10^{-5}$ M. Similarly, the binding of warfarin-^{14}C to albumin was measured at concentrations of the drug ranging from $1{\cdot}0 \times 10^{-5}$ to $3{\cdot}0 \times 10^{-5}$ M.

In preliminary experiments, it was determined that neither warfarin nor phenylbutazone was bound to the dialysis membrane over the range of concentrations studied.

In certain studies the extent of binding of warfarin-^{14}C or phenylbutazone-^{14}C at various concentrations was measured in the presence of a fixed concentration of unlabeled drug or fatty acid. Drugs were added to the dialysis bags dissolved in 0·1 ml of buffer or 0·1 N NaOH; fatty acids were added in 0·1 ml ethyl alcohol. Control studies demonstrated that these volumes of NaOH and ethyl alcohol did not alter the extent of binding of the labeled compounds to albumin.

The formulation of Klotz[7] was used to determine the nature of the displacement of warfarin-^{14}C and phenylbutazone-^{14}C from albumin by a variety of other compounds.

RESULTS

Binding of phenylbutazone-^{14}C to human albumin

There is extensive binding of phenylbutazone-^{14}C to human albumin. A linear relationship was demonstrated between the reciprocal of the concentration of unbound phenylbutazone-^{14}C and the reciprocal of the moles of phenylbutazone-^{14}C bound per mole of albumin over the range of concentrations of drug studied (Fig. 1). Phenylbutazone-^{14}C binds to a single site on human albumin with an affinity constant of $1{\cdot}17 \times 10^{5}$.

Nature of the displacement of phenylbutazone-^{14}C from human albumin by various drugs

In the presence of warfarin ($9{\cdot}1 \times 10^{-5}$ M), less phenylbutazone-^{14}C was bound to albumin (Fig. 1). A common ordinate intercept indicates that phenylbutazone-^{14}C and warfarin compete for the same binding site on albumin.

The following drugs also competitively inhibit the binding of phenylbutazone-^{14}C to human albumin: chlorophenoxyisobutyric acid, $0{\cdot}93 \times 10^{-3}$ M (Fig. 2); tolbutamide, $3{\cdot}71 \times 10^{-4}$ M (Fig. 3); sulfamethoxypyridazine, $7{\cdot}15 \times 10^{-4}$ M (Fig. 4); and indomethacin, $5{\cdot}61 \times 10^{-4}$ M (Fig. 5).

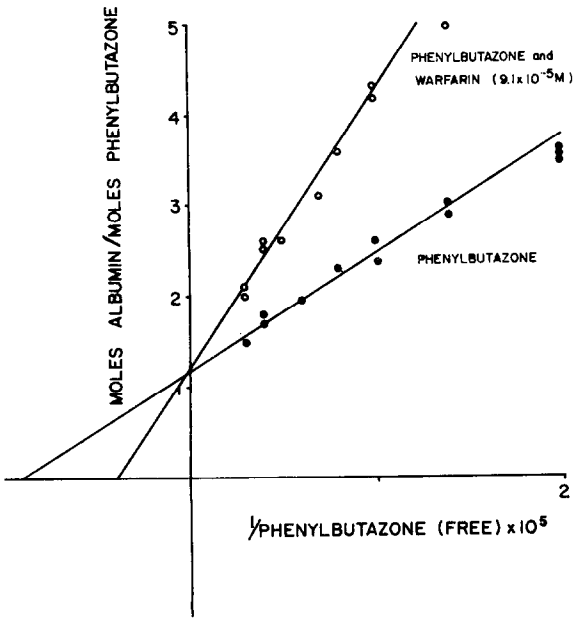

FIG. 1. The effect of warfarin on the binding of phenylbutazone-^{14}C to human albumin. The number in parentheses is the final concentration of warfarin used in all experiments.

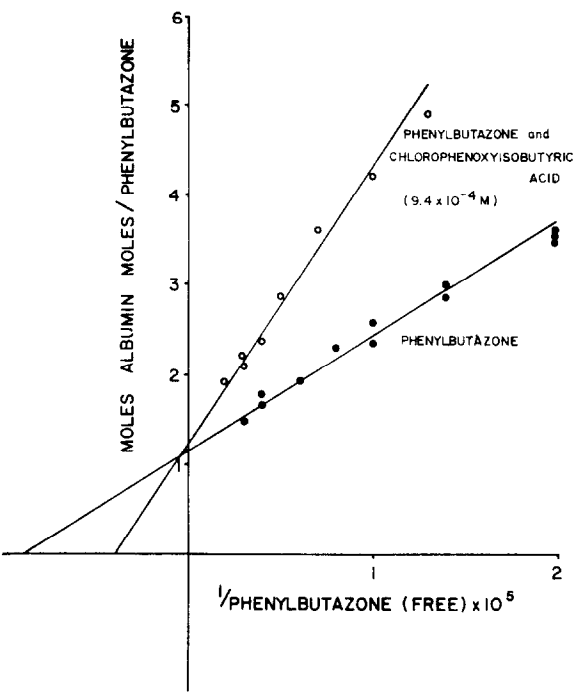

FIG. 2. The effect of CPIB on the binding of phenylbutazone-^{14}C to human albumin. The number in parentheses is the final concentration of CPIB used in all experiments.

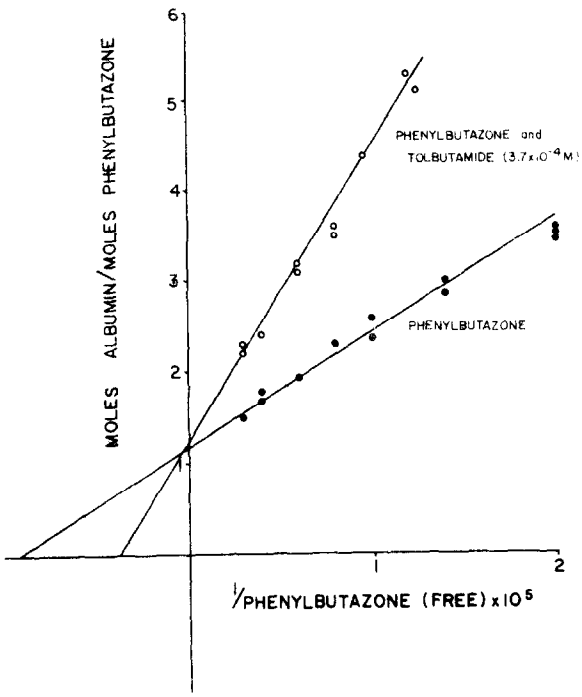

FIG. 3. The effect of tolbutamide on the binding of phenylbutazone-^{14}C to human albumin. The number in parentheses is the final concentration of tolbutamide used in all experiments.

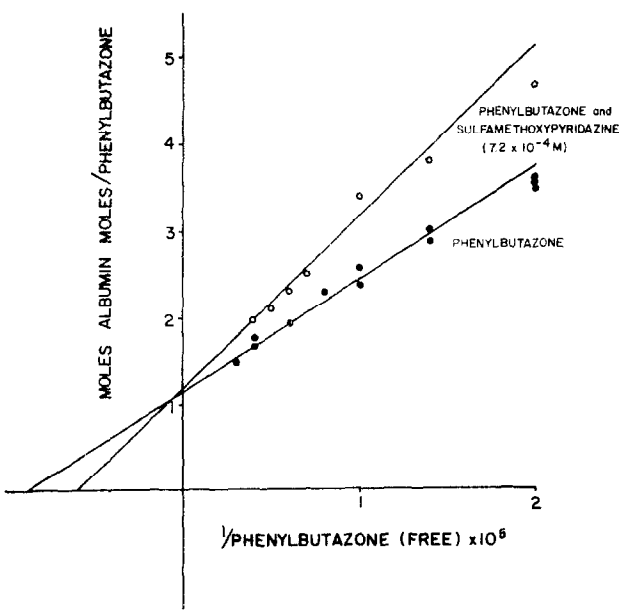

FIG. 4. The effect of sulfamethoxypyridazine on the binding of phenylbutaozne-^{14}C to human albumin. The number in parentheses is the final concentration of sulfamethoxypryidazine used in all experiments.

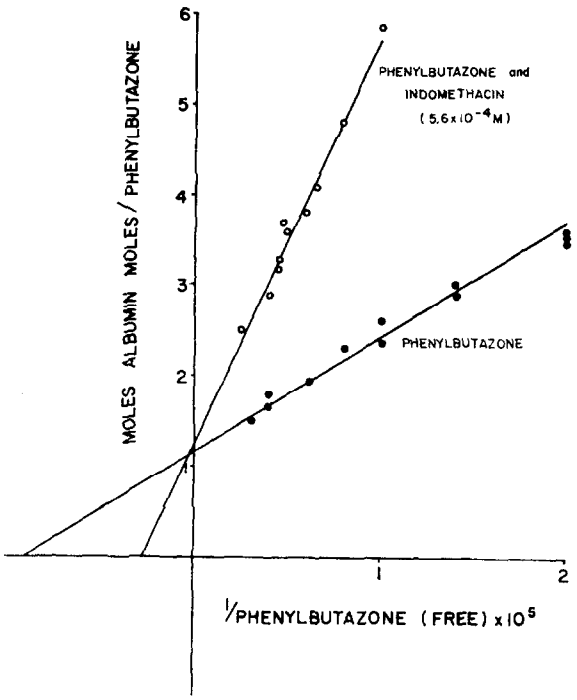

FIG. 5. The effect of indomethacin on the binding of phenylbutazone-^{14}C to human albumin. The number in parentheses is the final concentration of indomethacin used in all experiments.

Effect of various fatty acids on the binding of phenylbutazone-^{14}C and warfarin-^{14}C to human albumin

Stearic acid, myristic acid, and lauric acid decrease the extent of binding of phenylbutazone-^{14}C and warfarin-^{14}C to human albumin (Table 1). Warfarin-^{14}C was more easily displaced from albumin by these fatty acids than was phenylbutazone-^{14}C. The greatest displacement of both warfarin-^{14}C and phenylbutazone-^{14}C from human albumin occurred in the presence of lauric acid.

TABLE 1. EFFECT OF FATTY ACIDS ON THE BINDING OF WARFARIN-^{14}C AND PHENYLBUTAZONE-^{14}C TO HUMAN ALBUMIN*

Fatty acid	Warfarin-^{14}C (% bound)	Phenylbutazone-^{14}C (% bound)
	82·9 ± 1·6 (9)	91·3 ± 6·3 (9)
Stearic $3{\cdot}5 \times 10^{-3}$M	51·5 ± 2·2 (4)	67·7 ± 1·3 (4)
Myristic $3{\cdot}5 \times 10^{-3}$M	30·6 ± 1·7 (4)	50·9 ± 1·3 (4)
Lauric $3{\cdot}5 \times 10^{-3}$M	16·0 ± 1·1 (6)	28·6 ± 0·8 (6)

* In all experiments the final concentration of warfarin-^{14}C or phenylbutazone-^{14}C was $0{\cdot}041 \times 10^{-3}$M, and the final concentration of albumin was $10{\cdot}1 \times 10^{-5}$M. Results are the mean ± S.D. of 4–9 determinations. Figures in parentheses indicate the number of individual experiments.

Nature of the displacement of phenylbutazone-^{14}C and warfarin-^{14}C from human albumin by lauric acid

Lauric acid ($8{\cdot}8 \times 10^{-4}$ M) competitively inhibits the binding of phenylbutazone-^{14}C to human albumin (Fig. 6). At a concentration of $1{\cdot}8 \times 10^{-4}$ M, lauric acid competes with warfarin-^{14}C for the same binding site on the protein (Fig. 7).

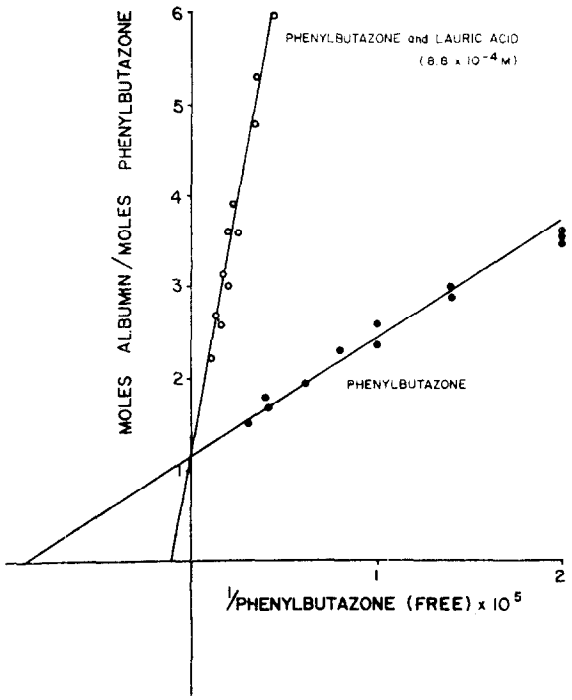

FIG. 6. The effect of lauric acid on the binding of phenylbutazone-^{14}C to human albumin. The number in parentheses is the final concentration of lauric acid used in all experiments.

Nature of the displacement of warfarin-^{14}C from human albumin by chlorophenoxyisobutyric acid

Chlorophenoxyisobutyric acid decreases the binding of warfarin-^{14}C to human albumin (Fig. 8). The common ordinate intercept indicates that chlorophenoxyisobutyric acid, at a concentration of $4{\cdot}6 \times 10^{-4}$ M, competes with warfarin-^{14}C for a common binding site on albumin.

DISCUSSION

The pharmacologic activity of a drug is dependent upon the concentration of drug at its cellular receptor site. When a drug is extensively bound to albumin, its pharmacologic activity may be influenced by factors which change the extent of binding of the drug.[1–3] Binding is affected by the plasma concentration of the drug or the presence of other drugs in the plasma which also bind to albumin.

Phenylbutazone is extensively bound to ablumin.[8] The binding capacity of normal human plasma for phenylbutazone is about 200 μg/ml. Burns *et al.* have demonstrated that the rate of metabolism of this drug is higher at plasma levels of about 200 μg/ml than at lower levels.[8] Presumably the increased rate of metabolism of

phenylbutazone at high concentrations of the drug is due to increased amounts of free drug available at the site of metabolism.

Warfarin is 97 per cent bound to plasma in man.[9] Previous studies have demonstrated that phenylbutazone displaces warfarin from its binding site on albumin.[3,5] The increased anticoagulant effect observed *in vivo* when warfarin and phenylbutazone are given concurrently may be attributed to an increase in concentration of free warfarin at its cell receptor site.

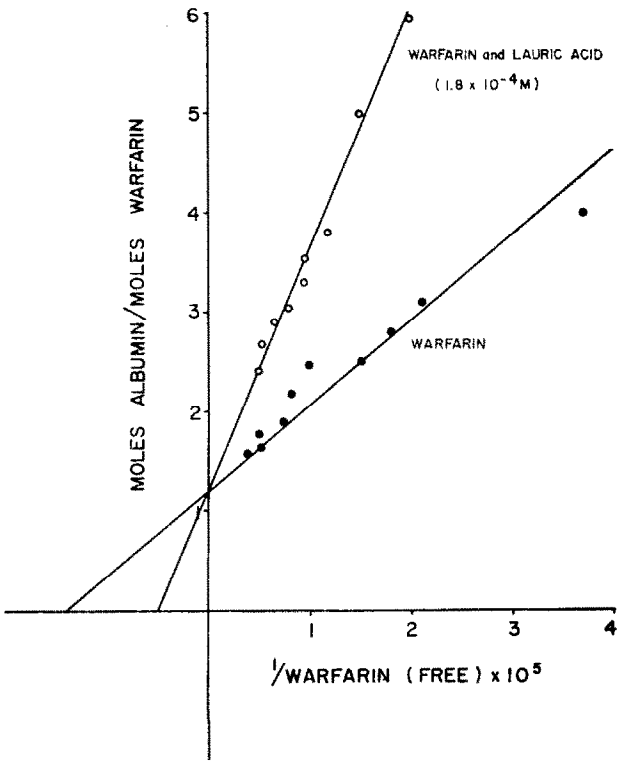

FIG. 7. The effect of lauric acid on the binding of warfarin-^{14}C to human albumin. The number in parentheses is the final concentration of lauric acid used in all experiments.

The present study demonstrates that warfarin is a competitive inhibitor of the binding of phenylbutazone-^{14}C to human albumin. Since phenylbutazone competitively inhibits the binding of warfarin-^{14}C to human albumin, both drugs must bind to the same site on the protein.

CPIB,[10] tolbutamide,[11] sulfamethoxypyridazine,[10] and indomethacin[12] are all extensively bound to albumin. In the present study these drugs displaced phenylbutazone-^{14}C from albumin by competing with it for the same binding site on the protein. Since phenylbutazone ($K = 1{\cdot}17 \times 10^5$) is more avidly bound to albumin than is warfarin ($K = 0{\cdot}88 \times 10^5$), it is likely that CPIB, tolbutamide, sulfamethoxypyridazine, and indomethacin will also displace warfarin from albumin. Indeed, in the present study CPIB competitively displaced warfarin-^{14}C from albumin. The nature of the displacement seems to depend upon the concentration of CPIB. In a

previous study with a lower concentration of CPIB, a noncompetitive type of displacement of the anticoagulant was demonstrated.[3] These observations suggest that CPIB binds to more than one type of site on the albumin molecule. This is not unexpected since other fatty acids are known to bind to at least three types of sites on the protein.[13]

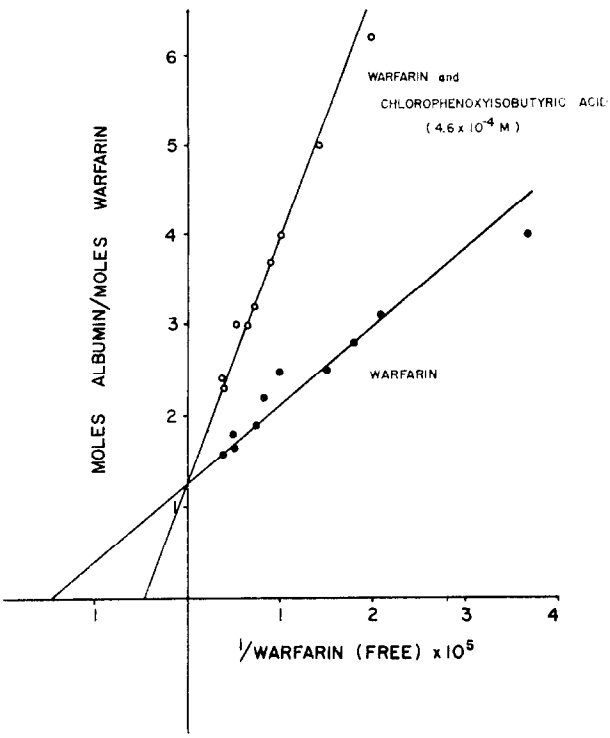

FIG. 8. The effect of CPIB on the binding of warfarin-^{14}C to human albumin. The number in parentheses is the final concentration of CPIB used in all experiments.

Fatty acids are much more avidly bound to albumin than either phenylbutazone or warfarin.[13] In the present study three fatty acids, lauric, myristic and stearic acid, were shown to displace both phenylbutazone and warfarin from albumin. Warfarin-^{14}C was more readily displaced than was phenylbutazone-^{14}C, confirming the previous observation that phenylbutazone is more avidly bound to the protein. Lauric acid was the most effective displacer of both drugs from albumin; stearic acid was the least effective. These results suggest that lauric acid is more avidly bound to albumin than is stearic acid. However, Boyer *et al.*[14] have demonstrated that the affinity of fatty acids for binding sites on albumin increases with increasing chain length of the fatty acid. Under the conditions of the present study, lauric acid was more soluble than either myristic acid or stearic acid; it therefore more effectively displaced phenylbutazone and warfarin from albumin than did the other fatty acids.

This study indicates that many acidic compounds of unrelated chemical structure bind to a common site on the albumin molecule. These compounds are extensively

ionized at pH 7·4 and presumably are associated with a cationic site on the protein. Currently no information is available as to the nature of this binding site. The binding of certain azo dyes and detergents to albumin produces configurational changes in the protein molecule.[15, 16] Such changes in the structure of the protein may occur at the site where drugs bind to the molecule. Thus the binding site would lack specificity and could accommodate compounds of diverse structure.

Displacement of a drug from binding sites on albumin may produce either an increased pharmacologic effect or a toxic effect dependent upon the nature of the drug. The present study demonstrates that several commonly used drugs can displace warfarin and phenylbutazone from binding sites on albumin and thus potentially produce undesired clinical effects.

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