Effects of Phenylbutazone, Tolbutamide, and Clofibric Acid on Binding of Racemic Warfarin and Its Enantiomers to Human Serum Albumin

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Abstract □ The effect of phenylbutazone, tolbutamide, and clofibric acid on the binding of racemic warfarin and its enantiomers to human serum albumin was studied by equilibrium dialysis. Warfarin had one primary and two secondary binding sites on the albumin molecule. No difference in binding was detected at the primary binding site; the extent of *R*(+)-isomer binding at the secondary binding sites was 2.5 times greater than the corresponding *S*(−)-isomer binding. Phenylbutazone and warfarin appear to compete for the same primary binding site on the albumin molecule. Tolbutamide interferes with the binding of warfarin enantiomers at their secondary sites. Clofibric acid has a less pronounced effect on warfarin binding than does phenylbutazone or tolbutamide.

Keyphrases □ Phenylbutazone—effect on binding of racemic warfarin and its enantiomers to human serum albumin □ Tolbutamide—effect on binding of racemic warfarin and its enantiomers to human serum albumin □ Clofibric acid—effect on binding of racemic warfarin and its enantiomers to human serum albumin □ Warfarin—effect of phenylbutazone, tolbutamide, and clofibric acid on binding of racemate and enantiomers to human serum albumin

The pharmacokinetics of the warfarin isomers show similarities as well as dissimilarities. The two isomers exhibit similar absorption patterns (1) and do not differ significantly in their volumes of distribution (2–4). The *S*(−)-isomer is several times more potent in anticoagulant activity than the *R*(+)-form (1, 4), and it is eliminated primarily as its oxidation product, 7-hydroxywarfarin, while the *R*(+)-isomer is predominantly reduced and eliminated as warfarin alcohol (1, 3, 5). Although interindividual differences produce a wide range of plasma half-lives (6–8), the *S*(−)-isomer has a shorter half-life than the *R*(+)-isomer (1–4).

In the present investigation, the binding behavior of the isomers with human serum albumin was characterized by equilibrium dialysis to determine if there is a correlation between the free fraction of the isomers *in vitro* and clinically observed elimination kinetics. In two studies (9, 10), the *S*(−)-isomer had a significantly higher affinity for the primary site; in two other studies (11, 12), no significant differences in isomer binding were found.

Phenylbutazone (13–18), tolbutamide (19), and clofibrate (17, 20, 21) have been reported to potentiate the anticoagulant effects of warfarin. Extensive work has been done on the phenylbutazone–warfarin interaction, including several clinical studies with the isomers (1, 22). Analysis of the elimination products during warfarin–phenylbutazone treatment provided interesting results: the clearance of *R*(+)-warfarin was stimulated while that of the *S*(−)-isomer was retarded (1). The greater prothrombin time response observed in these cases can be attributed to a combination of two effects: increased free warfarin concentration and inhibition of the metabolism and elimination of the more potent *S*(−)-isomer.

In view of the stereoselective interaction suggested by these findings, the effects of phenylbutazone, tolbutamide, and clofibric acid on the binding of racemic warfarin and its isomers to human serum albumin *in vitro* were studied. Since clofibrate undergoes hydrolysis *in vivo* to its corresponding acid, which is presumed to be the active drug (23), clofibric acid was used. Although binding parameters have been reported for phenylbutazone (24, 25), tolbutamide (26–28), and clofibric acid (29) under various conditions, they were experimentally estimated under the same conditions used here for comparison.

Table I—Estimated Binding Parameters

Compound	n_1	$k_1 \times 10^{-5}\ M^{-1}$	n_2	$k_2 \times 10^{-4}\ M^{-1}$
Racemic warfarin	1	14	2	1.8
R(+)-Warfarin	1	14	2	2.5
S(−)-Warfarin	1	14	2	1.0
Phenylbutazone	1	7.0	1	1.0
Tolbutamide	1	1.3	2	0.25
Clofibric acid	1	0.17	2	0.43

EXPERIMENTAL

Materials—The warfarin isomers[1], tolbutamide[2], clofibric acid[3], and phenylbutazone[4] were obtained commercially. Human serum albumin, rechromatographed, was used without further purification[5]. Dialysis membranes[6] were rinsed with distilled water several times during the 2 hr prior to use. Chloroform and dimethylformamide were spectrophotometric quality[7]. All other chemicals were certified ACS grade. All solutions were prepared in 0.067 *M* mixed sodium and potassium phosphate buffer, pH 7.4 (30).

Dialysis Procedure—Dialysis experiments were carried out at 4° in cells constructed from 30- and 60-ml glass jars attached by Plexiglas flanges. Cells were rotated at 6 rpm for ~135 hr. One compartment contained the drug (or drugs) in 30.0 ml of buffered 0.4% human serum albumin, and the other compartment contained 60.0 ml of buffer. A larger volume was used in the albumin-free compartment to increase the amount of free drug available for extraction and analysis. Cells without drugs were used as spectrophotometric blanks in the assay and to check for albumin transfer across the membrane.

Analytical Methods—The purity of the warfarin isomers, phenylbutazone, and tolbutamide was determined by comparison to their corresponding USP–NF reference standards. Clofibric acid purity was verified by titration. Optical rotation[8] of the warfarin enantiomers was measured to prove their optical purity: $[\alpha]_D^{25}$ +149.4° (c 1.2, 0.5 *M* NaOH) for *R*(+)-warfarin, and $[\alpha]_D^{25}$ −148.4° (c 1.2, 0.5 *M* NaOH) for *S*(−)-warfarin. Fluorescence[9] intensity of warfarin at 410 nm, excited at 327 nm, was linear over the standard concentration range and was unaffected by phenylbutazone, tolbutamide, or clofibric acid.

The warfarin–albumin solutions for dialysis were used as calibration standards for warfarin determinations on the albumin side after dialysis.

[1] Gift of Endo Laboratories, Garden City, N.Y.
[2] Gift of The Upjohn Co., Kalamazoo, Mich.
[3] Gift of Ayerst Laboratories, New York, N.Y.
[4] Sigma Chemical Co., St. Louis, Mo.
[5] Worthington Biochemical Corp., Freehold, N.J.
[6] Spectra/Por 2, Spectrum Medical Industries, Los Angeles, Calif.
[7] Burdick & Jackson Laboratories, Muskegon, Mich.
[8] Model A polarimeter, Bellingham-Stanley Ltd., London, England.
[9] Model RRS 1000 spectrofluorometer, Schoeffel Instrument Corp., Westwood, N.J.

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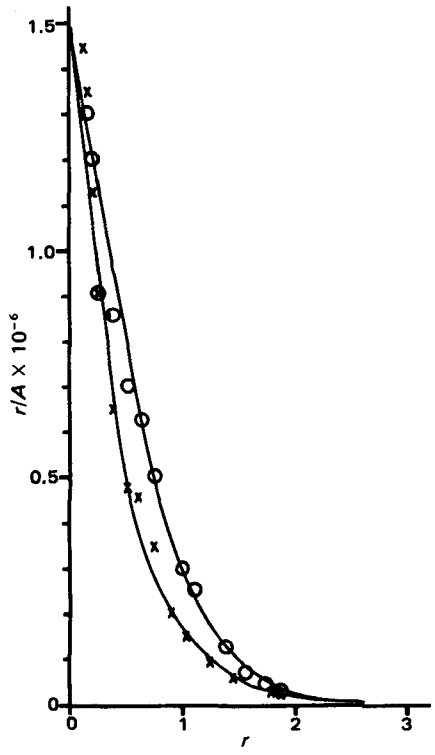

Figure 1—*Binding of warfarin enantiomers to albumin in 0.067* M *phosphate buffer, pH 7.4, at 4°. The smooth curve fitted to each set of points is based on the values in Table I. Key:* O, R(+)-*warfarin;* ×, S(−)-*warfarin;* r, *number of moles of warfarin bound per mole of human serum albumin; and* A, *molar concentration of free warfarin.*

Five milliliters of the standard and dialyzed solutions were diluted to 100 ml with dimethylformamide, and the resulting precipitate of buffer salts and albumin was allowed to settle overnight prior to fluorescence analysis of the clear supernate. The free warfarin side of the cell was treated similarly except for the lower levels, which were acidified, extracted with chloroform, converted to a residue, and dissolved in a minimum volume of dimethylformamide. Standard warfarin solutions were prepared to encompass the range of experimental free warfarin values.

The concentration of free phenylbutazone was determined from the absorbance of the dialysate at 264 nm[10], and the concentration of free tolbutamide was similarly determined at 227 nm. Clofibric acid levels were determined either from the solution absorbance at 228 nm or by GLC[11]. Clofibric acid estimation was not attempted in the presence of warfarin because of significant interference from warfarin.

For GLC, aliquots of the dialysate were acidified, extracted with chloroform, and converted to a residue. The residue was treated with 30 μl of bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane[12] and then diluted with 5.00 ml of internal standard solution consisting of 6.5 μl of heptadecane/100 ml of carbon disulfide. The samples were stoppered and allowed to stand overnight at room temperature to ensure complete derivatization prior to injection onto a 1.2-m × 3.5-mm i.d. coiled glass column packed with 3.0% dimethyl silicone fluid[13] on flux-calcined diatomaceous earth[14], 100–120 mesh, at 170° with a nitrogen carrier. Injection and flame-ionization detector temperatures were 225°. Quantitation of clofibric acid was accomplished by comparison to standard solutions similarly treated.

Binding Constants—Three dialysis determinations, each consisting of seven concentrations of $R(+)$-, $S(-)$-, and racemic warfarin, were made over the range of 6×10^{-6}–1.0×10^{-4} M. Binding constants for the other drugs were estimated from dialysis solutions covering the following ranges: phenylbutazone, 1.5×10^{-5}–1.7×10^{-4} M; tolbutamide, 2.7×10^{-5}–1.8×10^{-4} M; and clofibric acid, 1.2×10^{-5}–2.1×10^{-4} M. Direct spectrophotometric methods were used for determination of phenylbutazone, tolbutamide, and clofibric acid concentrations.

Displacement Experiments—Duplicate runs for warfarin and each competing drug were performed. Fixed concentrations of the competing drug were studied with the warfarin concentration from 6×10^{-6} to 3×10^{-5} M (2.5–10 mg/liter). Final concentrations for the competing drugs were: phenylbutazone, 1.7×10^{-4} M (52 mg/liter); tolbutamide, 1.4×10^{-4} M (38 mg/liter); and clofibric acid, 1.1×10^{-4} M (24 mg/liter).

In all cases, the free competing drug concentration was measured in addition to both bound and free warfarin levels. Free phenylbutazone and free tolbutamide were determined spectrophotometrically; free clofibric acid was measured by GLC.

RESULTS AND DISCUSSION

Racemic warfarin was used as the control in all cases to relate the present work to prior investigations and as a check on results obtained for isomers and competing drugs.

Analytical methods were developed for accuracy and facility of determining small drug quantities. Dimethylformamide was chosen as the solvent for the fluorescence measurements of warfarin because of its ability to enhance the fluorescence intensity (31, 32). To ensure the accuracy of the warfarin data, the drug was measured on both sides of all dialysis membranes, and the recovery of total warfarin was verified. The overall mean recovery for 185 determinations of both free and bound warfarin, alone and in combination with other drugs, was 100 ± 2%.

Scatchard plots (33) are presented in Fig. 1 for the $R(+)$- and $S(-)$-isomers. Curvature is present in both cases, suggesting multiple binding sites. At low bound drug-to-total protein ratios ($r < 0.5$), there appears to be no significant difference in the binding of the two isomers; at higher binding levels ($r > 0.5$), a distinct difference is observed. This finding suggests either different secondary sites or stereoselectivity at identical sites. Estimates of binding constants made by computer-assisted successive approximations from the experimental curves are presented in Table I. The computer was used to generate r values for the Scatchard equations for two independent binding sites, using experimental free warfarin concentrations and varying values for the binding constants. Coincidence of fit was tested by visual comparison of the plotted experimental and computed values. The binding pattern observed for racemic warfarin and the constants determined reflect the average behavior of the isomers. Primary constants were the same for both isomers, but secondary binding constants were quite different, with the value for the $R(+)$-isomer (2.5×10^4) being significantly larger than the corresponding value for the $S(-)$-isomer (1.0×10^4).

Binding parameters for the competing drugs were experimentally estimated for comparison. Curvature is evident in Scatchard plots for all drugs, suggesting multiple sites with differing affinities. The estimated binding parameters for these drugs, obtained by computer-assisted successive approximations from the experimental curves, are presented in Table I.

Duplicate runs for warfarin and each competing drug were performed to evaluate the possible effect of addition order on the binding behavior. In the first case, the warfarin–albumin solutions were allowed to equilibrate overnight at 4°, the competing drug was added, and the solutions were dialyzed. In the second case, the competing drug–albumin solutions were allowed to equilibrate before warfarin addition. Equilibrium concentrations for all drugs were essentially the same in both runs. This finding suggests that, within the ranges studied, equilibrium concentrations of the drugs are a function of the concentrations and affinity constants of the particular drugs and are not related to the addition order.

The concentration of free competing drug was measured as well as bound and free warfarin to estimate the extent of competing drug binding and to correlate this information with the observed behavior for warfarin alone. The warfarin concentration was varied over the range where one binding site for warfarin would be primarily operative in the absence of competition. This range includes the therapeutic blood levels reported for warfarin (34). To minimize the drug-to-albumin ratio, the competing drug concentration added was adjusted so that the final total competing drug concentration at equilibrium would represent about half the average blood levels reported for these drugs (35–37).

Since the Klotz plot (38) is one of the best graphical representations of binding over a narrow concentration range, this treatment was chosen for data presentation (Figs. 2–5). Values for these figures are averages of two runs with reverse initial equilibration as previously described. Apparent primary binding constants for warfarin in the presence of the three competing drugs are presented in Table II. Since no curvature was

[10] Model 200 UV-visible spectrophotometer, Perkin-Elmer Corp., Norwalk, Conn.
[11] Model 5840 gas chromatograph, Hewlett-Packard, Paramus, N.J.
[12] Regisil, RC-2, Regis Chemical Co., Morton Grove, Ill.
[13] OV-101, Ohio Valley Specialty Chemical, Marietta, Ohio.
[14] Gas Chrom Q, Applied Science Laboratories, State College, Pa.

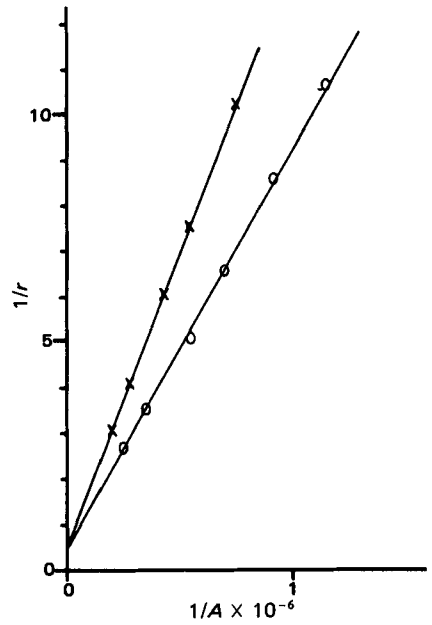

Figure 2—*Effect of phenylbutazone, 1.7 × 10⁻⁴ M, on binding of warfarin enantiomers to albumin. The warfarin concentration range was 6 × 10⁻⁶–3 × 10⁻⁵ M. Key:* O, R(+)-*warfarin;* ×, S(−)-*warfarin;* r, *number of moles of warfarin bound per mole of human serum albumin; and* A, *molar concentration of free warfarin.*

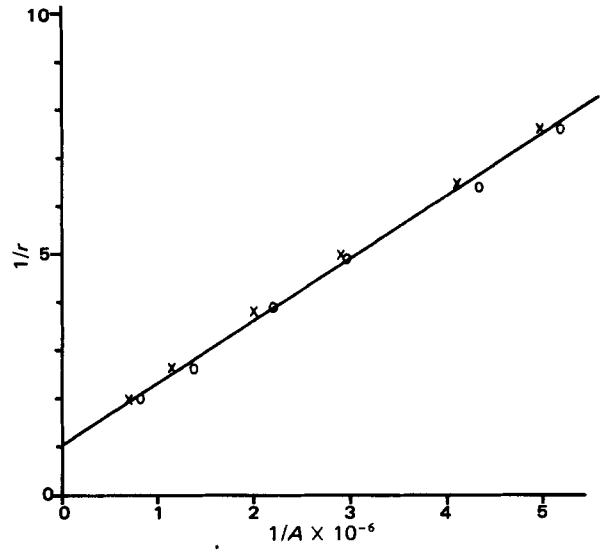

Figure 4—*Effect of clofibric acid, 1.1 × 10⁻⁴ M, on binding of warfarin enantiomers to albumin. The warfarin concentration range was 6 × 10⁻⁶–3 × 10⁻⁵ M. Key:* O, R(+)-*warfarin;* ×, S(−)-*warfarin;* r, *number of moles of warfarin bound per mole of human serum albumin; and* A, *molar concentration of free warfarin.*

evident in the Klotz plots, linear regression analysis was used to estimate the apparent binding parameters for warfarin in the presence of the competing drugs.

A marked increase in free warfarin was observed when phenylbutazone was present (14–20% *versus* 1–3% free warfarin without phenylbutazone). An r value of 1.57 was determined for phenylbutazone, indicating that it was occupying most of its primary sites as well as a significant number of its secondary sites. The apparent number of binding sites for warfarin, three, suggests substantial use of secondary binding sites. The apparent binding constant for the $R(+)$-isomer was significantly larger than the apparent binding constant for the $S(-)$-isomer, which is consistent with secondary site binding and with the significant differences observed in the free warfarin level between the two isomers [14–16% for the $R(+)$ *versus* 20–22% for the $S(-)$].

This difference in isomer behavior is clearly evident in the Klotz plot in Fig. 2. In view of the observed behavior, it appears that phenylbutazone and warfarin compete for the same primary binding site. With most primary sites occupied by phenylbutazone, warfarin binding is essentially limited to the secondary sites. This behavior is substantiated by the observed higher percentage of free $S(-)$-isomer. It is unlikely that phenylbutazone and warfarin also compete for the same secondary sites since, with r = 1.57 for phenylbutazone, the level of free warfarin would be higher than observed.

A significant increase in percent free warfarin was seen when tolbu-

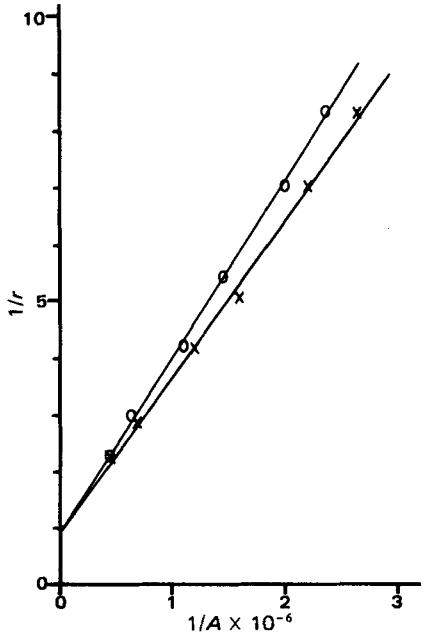

Figure 3—*Effect of tolbutamide, 1.4 × 10⁻⁴ M, on binding of warfarin enantiomers to albumin. The warfarin concentration range was 6 × 10⁻⁶–3 × 10⁻⁵ M. Key:* O, R(+)-*warfarin;* ×, S(−)-*warfarin;* r, *number of moles of warfarin bound per mole of human serum albumin; and* A, *molar concentration of free warfarin.*

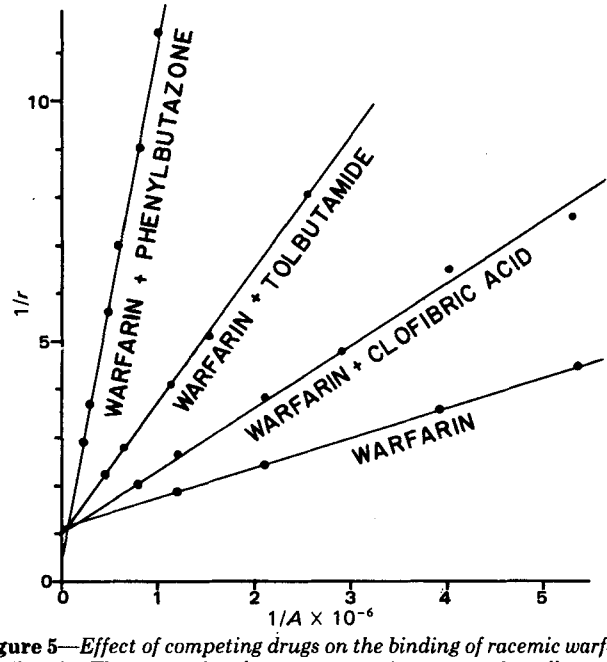

Figure 5—*Effect of competing drugs on the binding of racemic warfarin to albumin. The competing drug concentrations were: phenylbutazone, 1.7 × 10⁻⁴ M; tolbutamide, 1.4 × 10⁻⁴ M; and clofibric acid, 1.1 × 10⁻⁴ M. The warfarin concentration range was 6 × 10⁻⁶–3 × 10⁻⁵ M. Key:* r, *number of moles of warfarin bound per mole of human serum albumin; and* A, *molar concentration of free warfarin.*

Table II—Apparent Binding Parameters for Warfarin and Its Enantiomers in the Presence of Competing Drugs

Competing Drug	Racemic Warfarin		*R*(+)-Warfarin		*S*(−)-Warfarin	
	n	$k \times 10^{-5}$ M^{-1}	*n*	$k \times 10^{-5}$ M^{-1}	*n*	$k \times 10^{-5}$ M^{-1}
Phenylbutazone	2.9	0.32	2.8	0.40	3.4	0.22
Tolbutamide	1.1	3.1	1.3	2.4	1.1	3.5
Clofibric acid	0.85	9.5	0.89	9.2	0.89	8.6

tamide was added to the system (5–8% *versus* 1–3% free warfarin without tolbutamide). The *r* value was determined to be about one, indicating that tolbutamide was occupying a significant number of its primary sites. Therefore, it is unlikely that tolbutamide and warfarin compete for the same primary site since the increase in free warfarin would have been larger, similar to the case of phenylbutazone. Also, the apparent *n* for warfarin in the presence of tolbutamide was about one, indicating that it was binding predominantly at its primary site.

Tolbutamide binding probably affects the secondary sites for warfarin. The additional free warfarin observed was in the same range as the contribution of the secondary sites to warfarin binding (4–7%). Warfarin behavior, however, was anomalous (Fig. 3). The apparent primary affinity constant for the *S*(−)-isomer was significantly larger than that observed for the *R*(+)-isomer, resulting in a reversal of the relative amounts of free isomers. Tolbutamide binding may cause a conformational change in the albumin molecule, which results in a change in the affinities of the stereoselective secondary sites for the warfarin enantiomers.

The increase in free warfarin in the presence of clofibric acid was less than that found with either phenylbutazone or tolbutamide (3–5% *versus* 1–3% free warfarin without clofibric acid). Figure 4 shows essentially no difference in the binding behavior of the isomers. The *r* value of about one indicates that clofibric acid was occupying a significant number of its primary sites. Therefore, it is unlikely that clofibric acid and warfarin compete for the same primary site since the increase in free warfarin would have been larger. Also, the apparent *n* for warfarin in the presence of clofibric acid was about one, indicating that it was binding principally at its primary site. Some interaction at the secondary sites may be occurring.

In all cases, the behavior of free warfarin reflected the average behavior for the two isomers. To depict the relative effects of the three competing drugs, the data obtained for racemic warfarin alone and in combination with each competing drug were composited (Fig. 5). The relative magnitude of their effects was proportional to their relative affinity constants for human serum albumin. Significant effects were observed when phenylbutazone, with a similar primary affinity constant to that of warfarin, acted as the competing drug. Less pronounced but potentially significant effects were demonstrated with tolbutamide and clofibric acid. These findings indicate that interactions at the albumin binding sites may contribute to the reported potentiating effects of these drugs *in vivo*.

Significant correlations have been demonstrated between the free fraction and total body clearance of racemic warfarin (39, 40). This correlation has been predicted on theoretical grounds and indicates that serum protein binding is a major determinant of the elimination kinetics of warfarin and of interindividual variations in its body clearance. Since no difference in binding was detected at the primary site, the corresponding free fractions for the isomers at this site do not correlate with warfarin elimination kinetics. The greater free fraction of the *S*(−)-isomer resulting from differences in secondary site binding correlates with its clinically observed shorter plasma half-life. This finding suggests that binding at the secondary sites may be more significant *in vivo* due to endogenous substances (such as free fatty acids), which have been reported to compete with warfarin for its primary binding site (24, 41).

REFERENCES

(1) R. J. Lewis, W. F. Trager, K. K. Chan, A. Breckenridge, M. Orme, M. Roland, and W. Schary, *J. Clin. Invest.*, **53**, 1607 (1974).

(2) R. A. O'Reilly, *Clin. Pharamcol. Ther.*, **16**, 348 (1974).

(3) D. S. Hewick and J. McEwen, *J. Pharm. Pharmacol.*, **25**, 458 (1973).

(4) A. Breckenridge, M. Orme, H. Wesseling, R. J. Lewis, and R. Gibbons, *Clin. Pharmacol. Ther.*, **15**, 424 (1974).

(5) K. K. Chan, R. J. Lewis, and W. F. Trager, *J. Med. Chem.*, **15**, 1265 (1972).

(6) A. Yacobi, L. B. Wingard, and G. Levy, *J. Pharm. Sci.*, **63**, 868 (1974).

(7) R. A. O'Reilly, P. M. Aggeler, M. S. Hoag, and L. Leong, *Thromb. Diath. Haemorrh.*, **8**, 82 (1962).

(8) L. B. Wingard, Jr., R. A. O'Reilly, and G. Levy, *Clin. Pharmacol. Ther.*, **23**, 212 (1978).

(9) J. H. M. Miller and G. A. Smail, *J. Pharm. Pharmacol.*, **29**, 33P (1977).

(10) E. M. Sellers and J. Koch-Weser, *Pharmacol. Res. Commun.*, **7**, 331 (1975).

(11) N. A. Brown, E. Jähnchen, W. E. Müller, and U. Wollert, *Mol. Pharmacol.*, **13**, 70 (1977).

(12) R. A. O'Reilly, *ibid.*, **7**, 209 (1971).

(13) S. Chierichetti, G. Bianchi, and B. Cerri, *Curr. Ther. Res. Clin. Exp.*, **18**, 568 (1975).

(14) W. L. Schary, R. J. Lewis, and M. Rowland, *Res. Commun. Chem. Pathol. Pharmacol.*, **10**, 663 (1975).

(15) R. A. O'Reilly and P. M. Aggeler, *Proc. Soc. Exp. Biol. Med.*, **128**, 1081 (1968).

(16) P. M. Aggeler, R. A. O'Reilly, L. Leong, and P. E. Kowitz, *N. Engl. J. Med.*, **276**, 496 (1967).

(17) J. A. Udall, *Clin. Med.*, **77**, 20 (1970).

(18) R. A. O'Reilly, *Ann. N.Y. Acad. Sci.*, **226**, 293 (1973).

(19) E. W. Martin, "Hazards of Medication," Lippincott, Philadelphia, Pa., 1971, pp. 470–484.

(20) R. A. O'Reilly, M. A. Sahud, and A. J. Robinson, *Thromb. Diath. Haemorrh.*, **27**, 309 (1972).

(21) S. M. Pond, G. G. Graham, N. D. Wade, and G. Sudlow, *Aust. N.Z. J. Med.*, **5**, 324 (1975).

(22) R. A. O'Reilly, *J. Am. Med. Assoc.*, **238**, 574 (1977).

(23) J. M. Thorp, *Lancet*, **1**, 1323 (1962).

(24) M. H. Solomon, J. J. Schrogie, and D. Williams, *Biochem. Pharmacol.*, **17**, 143 (1968).

(25) C. F. Chignell, *Mol. Pharmacol.*, **5**, 224 (1969).

(26) M. J. Crooks and K. F. Brown, *J. Pharm. Sci.*, **62**, 1904 (1973).

(27) P. Hsu, J. K. H. Ma, and L. A. Luzzi, *ibid.*, **63**, 570 (1974).

(28) J. Judis, *ibid.*, **61**, 89 (1972).

(29) R. I. Nazareth, T. D. Sokoloski, D. T. Witiak, and A. T. Hopper, *ibid.*, **63**, 203 (1974).

(30) I. M. Kolthoff and C. Rosenblum, "Acid-Base Indicators," Macmillan, New York, N.Y., 1937, p. 249.

(31) C. F. Chignell, *Ann. N.Y. Acad. Sci.*, **226**, 44 (1973).

(32) K. A. Bachmann and A. M. Burkman, *J. Pharm. Pharmacol.*, **27**, 832 (1975).

(33) G. Scatchard, *Ann. N.Y. Acad. Sci.*, **51**, 660 (1949).

(34) P. B. Andreasen and E. S. Vesell, *Clin. Pharmacol. Ther.*, **16**, 1059 (1974).

(35) J. J. Burns, R. K. Rose, T. Chenkin, A. Goldman, A. Schulert, and B. B. Brodie, *J. Pharmacol. Exp. Ther.*, **109**, 346 (1953).

(36) S. B. Matin, S. H. Wan, and J. H. Karam, *Clin. Pharmacol. Ther.*, **16**, 1052 (1974).

(37) T. Taylor and L. F. Chasseaud, *J. Pharm. Sci.*, **66**, 1639 (1977).

(38) I. M. Klotz and F. M. Walker, *J. Am. Chem. Soc.*, **70**, 943 (1948).

(39) A. Yacobi, J. A. Udall, and G. Levy, *Clin. Pharmacol. Ther.*, **19**, 552 (1976).

(40) A. Yacobi, T. Lampman, and G. Levy, *ibid.*, **21**, 286 (1977).

(41) O. G. Nelson, L. Storstein, and S. Jacobsen, *Biochem. Pharmacol.*, **26**, 229 (1977).

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