REFERENCES

(1) R. F. Doerge, in "Textbook of Organic Medicinal and Pharmaceutical Chemistry," C. O. Wilson, O. Gisvold, and F. R. Doerge, Eds., 6th ed., J. B. Lippincott, Philadelphia, Pa., 1971, p. 649.

(2) W. Voegtli, U.S. pat. 2,857,383 (1958); *Chem. Abstr.*, **53**, 6249h (1959).

(3) R. W. Goettsch and G. A. Wiese, *J. Am. Pharm. Assoc., Sci. Ed.*, **47**, 319 (1958).

(4) E. Campaigne and T. Bosin, *J. Med. Chem.*, **10**, 945 (1967).

(5) A. Shafiee, M. Vossoghi, J. Wossooghi, and S. Yazdani, *J. Pharm. Sci.*, **70**, 566 (1981).

(6) A. Shafiee, F. Savabi, A. Rezvani, M. Farrokhsiar, and A. Khoyi, *ibid.*, **67**, 125 (1978).

(7) C. S. Weil, *Biometrics*, **8**, 243 (1952).

(8) M. R. A. Chance and H. J. Lobstein, *J. Pharmacol. Exp. Ther.*, **82**, 203 (1944).

(9) A. H. Campbell, J. A. Strasse, G. H. Lord, and J. E. Wilson, *J. Pharm. Sci.*, **57**, 2045 (1968).

(10) J. Magnus, *Pflugers Arch. Ges. Physiol.*, **102**, 123 (1904).

(11) G. Komppa and S. Weckman, *J. Prakt. Chem.*, **138**, 109 (1933).

ACKNOWLEDGMENTS

Supported by a grant from the Research Council of the University Crusade of Tehran University.

The authors thank Drs. M. A. Khoyi and K. Mohammad for their fruitful discussion.

Binding of Several Phenothiazine Neuroleptics to a Common Binding Site of α_1-Acid Glycoprotein, Orosomucoid

SAFAA EL-GAMAL *§, UWE WOLLERT *†, and WALTER E. MÜLLER ‡ ×

Received December 14, 1981, from the **Pharmakologie und Toxikologie für Naturwissenschaftler, Fachgereich Pharmazie, Universität Mainz* and the ‡*Pharmakologisches Institut der Universität Mainz, Obere Zahlbacher Strasse 67, D-6500 Mainz, West Germany.* Accepted for publication April 1, 1982. §Present address: Department of Pharmaceutics, Faculty of Pharmacy, Alexandria University, Alexandria, Egypt. †Deceased.

Abstract □ The interaction of several phenothiazine neuroleptics with α_1-acid glycoprotein was investigated using circular dichroism and equilibrium dialysis techniques. For chlorpromazine only, one high-affinity binding site of the protein was found. The binding of the drug to this single site generated typical polyphasic extrinsic Cotton effects. Since several other phenothiazine neuroleptics gave qualitatively comparable extrinsic Cotton effects in the presence of α_1-acid glycoprotein and potently inhibited the binding of chlorpromazine to the single site, it was concluded that all phenothiazine derivatives investigated bound preferentially to only one common binding site of the α_1-acid glycoprotein molecule.

Keyphrases □ Phenothiazines—neuroleptics, binding to a common binding site of α_1-acid glycoprotein, orosomucoid □ Neuroleptic agents—binding of phenothiazines to a common binding site of α_1-acid glycoprotein, orosomucoid □ α_1-Acid glycoproteins—binding of several phenothiazine neuroleptics to a common binding site, orosomucoid □ Orosomucoid—binding of several phenothiazine neuroleptics to a common binding site of α_1-acid glycoprotein

While for most neutral or anionic drugs the predominating role of the albumin fraction as the major binding component in human blood is established, increasing evidence has been presented during recent years that this is not the case for several basic drugs where other proteins also contribute considerably to the plasma binding. Out of these, orosomucoid (α_1-acid glycoprotein) received the most attention because of its possible significance for the pharmacokinetic pattern of basic drugs (1). Thus, large variations in the blood levels of α_1-acid glycoprotein observed in patients suffering from various disease states could have been responsible for similarly large variations of the free plasma levels of some basic drugs measured in the same patients (1–3). Since the average plasma levels of α_1-acid glycoprotein are rather low, usually between 10 and 40 μmole/liter (2, 3), a fairly strong drug binding to this protein has to be assumed if variations of its plasma levels were to contribute considerably to the free fraction of a drug. Some recent work shows that several basic drugs are bound very strongly to α_1-acid glycoprotein (4–8). An example of this is the phenothiazine derivative, perazine, which is bound with very high affinity to mainly one site of the α_1-acid glycoprotein molecule (5–7).

The present study reports similar findings for chlorpromazine. Evidence is presented that a variety of phenothiazine neuroleptics, including perazine and chlorpromazine, are preferentially, if not exclusively, bound to only one common binding site of the α_1-acid glycoprotein molecule.

EXPERIMENTAL

Materials—α_1-Acid glycoprotein[1] (orosomucoid) had an electrophoretic purity >99%. [^{14}C]Chlorpromazine[2] had a specific activity of 80 mCi/mmole and a radiochemical purity >99%. All chlorpromazine derivatives were gifts from the manufacturers[3]. All other chemicals were obtained from commercial suppliers. All solutions were prepared with deionized water.

Circular Dichroism Measurements—Circular dichroism measurements were carried out with a spectropolarimeter[4] calibrated with *d*-camphorsulfonic acid. All spectra were recorded in cylindrical cells with 10-mm path length using a full-scale deflection of 0.02° θ and a spectral band width of 2 nm. All measurements were made in 0.07 *M* phosphate buffer (pH 7.4). Results are expressed as molar ellipticity ($[\theta]$) calculated with reference to the α_1-acid glycoprotein concentration (25 μM).

Equilibrium Dialysis—Binding of [^{14}C]chlorpromazine to α_1-acid glycoprotein was determined by equilibrium dialysis using a protein concentration of 12.5 μM and varying concentrations of the drug. All solutions were prepared in 0.07 *M* phosphate buffer (pH 7.4); 0.9 ml of the protein solution was dialyzed for 16 hr in the dark against 0.9 ml of buffer containing [^{14}C]chlorpromazine. One-milliliter dialysis cells and

[1] Behringwerke, Marburg, West Germany.

[2] Amersham Buchler, Braunschweig, West Germany.

[3] Perazine from Promonta, Hamburg, West Germany, promazine from Wyeth, Münster, West Germany; prothipendyl from Homburg, Frankfurt, West Germany; trifluopromazine from Heyden, Munich, West Germany; acepromazine from Clin-Comar, Paris, France.

[4] Cary 61.

0022-3549/83/0200-0202$01.00/0

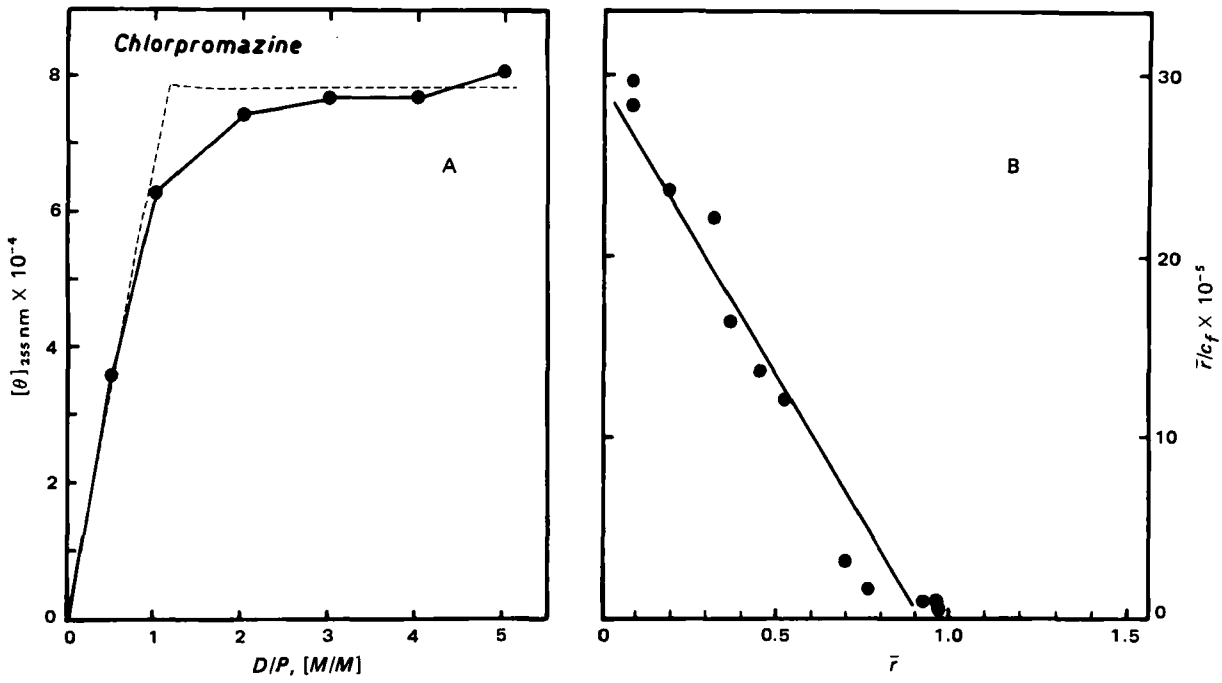


Figure 1—*The interaction of chlorpromazine with only one binding site of α_1-acid glycoprotein. (A) The relationship between the molar chlorpromazine–α_1-acid glycoprotein ratio (D/P) and the intensity of the induced circular dichroism band at 255 nm ($[\theta]_{255}$). (B) Scatchard plot of the interaction of [^{14}C]chlorpromazine with α_1-acid glycoprotein; k is the association constant (2.94×10^5); n is the number of binding sites per α_1-acid glycoprotein molecule (0.91); and r is the correlation coefficient of the calculated regression line (0.96).*

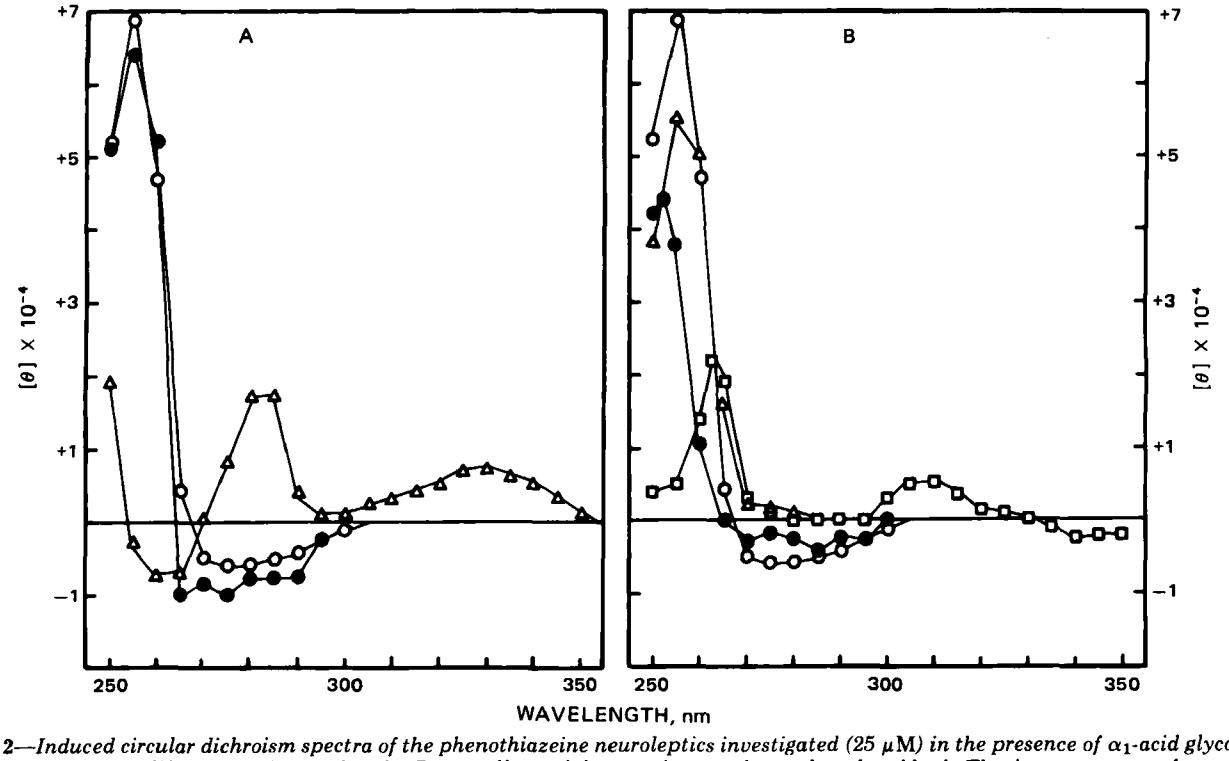


Figure 2—*Induced circular dichroism spectra of the phenothiazeine neuroleptics investigated (25 μM) in the presence of α_1-acid glycoprotein (25 μM). The data are difference values, using the Cotton effects of the protein at each wavelength as blank. The data are means of two different runs. Key: (A) (●) chlorpromazine; (○) promazine; (△) acepromazine; (B) (○) promazine; (●) prothipendyl; (△) perazine; (□) trifluoperazine.*

cellophane dialysis membranes[5] were used. The radioactivity at both sides of the membrane was determined by liquid scintillation spectrometry[6].

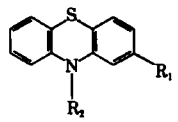
RESULTS AND DISCUSSION

Plotting the binding data obtained by the equilibrium dialysis experiments for the interaction of [^{14}C]chlorpromazine with α_1-acid glycoprotein according to Scatchard (9) revealed a straight line with an abscissa intercept of 0.9, indicating that [^{14}C]chlorpromazine binds within the concentration range investigated (up to a molar drug–protein ratio of 5) to only one site of the α_1-acid glycoprotein moleculer (Fig. 1B). As indicated by the association constant, k, $\sim 3 \times 10^6\ M^{-1}$, the affinity of this single site for chlorpromazine is very high and within the range of the strongest drug interactions with human serum albumin (10–12).

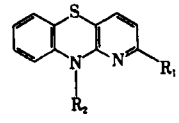
The binding of chlorpromazine to α_1-acid glycoprotein profoundly

[5] Union Carbide.
[6] Packard model 3255.

Table I—Structural Requirements for the High-Affinity Binding of Phenothiazine Neuroleptics to α_1-Acid Glycoprotein



I-VI



VII

Compound	R_1	R_2	log P^a	$\Delta\alpha^b$, %	λ_{max}^c, nm	$[\theta] \times 10^{4d}$ $D/P, M/M$ 1	2	3
Promazine (I)	—H	—CH_2—CH_2—CH_2—N(CH_3)(CH_3)	2.6	19	255	+6.8	+8.3	+8.6
Chlorpromazine (II)	—Cl	—CH_2—CH_2—CH_2—N(CH_3)(CH_3)	3.3	30	255	+6.3	+7.8	+8.2
Acepromazine (III)	—OC—CH_3	—CH_2—CH_2—CH_2—N(CH_3)(CH_3)	2.3	16	260	−0.8	−1.0	−1.0
Trifluopromazine (IV)	—CF_3	—CH_2—CH_2—CH_2—N(CH_3)(CH_3)	3.4	23	—	—	—	—
Perazine (V)	—H	—CH_2—CH_2—CH_2—N(piperazine)—CH_3	2.9	14	255	+5.2	+6.8	+7.6
Trifluoperazine (VI)	—CF_3	—CH_2—CH_2—CH_2—N(piperazine)—CH_3	4.75[e]	21	263	+2.4	+3.5	+3.8
Prothipendyl (VII)	—H	—CH_2—CH_2—CH_2—N(CH_3)(CH_3)	2.2	24	253	+4.1	+4.8	+5.4

[a] The partition coefficients P between n-octanol and buffer were taken from Refs. 14 and 15. [b] Increase of the percentage free (α) of [^{14}C]chlorpromazine (6.3 μM) in the presence of the unlabeled neuroleptics (25 μM). Without displacer the percentage free (α) was 9.0 ± 0.9 (n = 6). [c] Wavelength of the induced circular dichroism band. [d] Intensity of the induced circular dichroism band at various molar drug–protein (D/P) ratios. [e] Calculated from a linear relationship between partition coefficients of n-octanol and buffer and R_M values obtained from a reverse-phase thin-layer technique, Ref. 15.

changes the native intrinsic circular dichroism spectrum of the protein between 250 and 350 nm (4, 13). In the difference spectrum, when the intrinsic Cotton effects of the protein alone have been subtracted, typical polyphasic extrinsic Cotton effects can be seen (Fig. 2). For chlorpromazine, a large positive band around 255 nm and two weaker negative bands around 27 nm can be seen (Fig. 2). The change in intensity of all three bands depends on the drug–protein concentration ratio in a similar fashion which can be demonstrated using the data for the strong positive band at 255 nm (Fig. 1A). The results show that maximum induction of the Cotton effects occurs when the drug–protein ratio reaches 1, indicating that the induction of Cotton effects is associated with the binding of chlorpromazine to only one site.

Several other phenothiazine neuroleptics also generate extrinsic Cotton effects in the presence of α_1-acid glycoprotein which are qualitatively similar to those of chlorpromazine (Fig. 2). The two most pronounced exceptions are the presence of an additional positive band >300 nm in the case of acepromazine and trifluoperazine and the positive sign of the band of acepromazine around 270 nm, instead of the negative sign observed for all other derivatives (Fig. 2). For trifluopromazine no extrinsic Cotton effects up to a molar excess of five were observed. The extrinsic Cotton effects of all phenothiazine derivatives depend on the drug concentration in a manner similar to that of chlorpromazine as shown for the positive band around 260 nm (Table I). Thus, the induced Cotton effects of the five chlorpromazine derivatives may also be associated with one preferential binding site. Moreover, the qualitatively comparable spectra suggest that the steric parameters of these interactions are similar for the drugs investigated, which might indicate that all phenothiazine derivatives investigated share the same high-affinity binding site with chlorpromazine. In agreement with these assumptions are the findings that all derivatives investigated inhibit the binding of [^{14}C]chlorpromazine to the single binding site; however, there are different potencies (Table I). Thus, considering these observations and the findings of only one high-affinity binding site of perazine (5–7), the assumption of one common high-affinity binding site of α_1-acid glycoprotein for most phenothiazine neuroleptics is justified.

In contrast to the rather unspecific binding of phenothiazine neuroleptics to serum albumin where good correlations between hydrophobic character and binding have been reported (14, 15) there is neither a clear correlation between lipophilicity (indicated by the partition coefficients in Table I) and the potencies of the derivatives as inhibitors of [^{14}C]-chlorpromazine binding nor between lipophilicity and the intensities of the induced Cotton effects (Table I). Structural parameters other than the lipophilicity are more important. As far as it is possible with the limited data available at the present state, it seems that for the intensity of the extrinsic Cotton effects and for the binding itself (indicated by the increase of the free fraction of [^{14}C]chlorpromazine) the substituent —R_1 on the phenothiazine nucleus is much more important than the aliphatic side chain (—R_2). This is best seen when comparing the data for promazine with either the data of acepromazine or trifluopromazine or comparing promazine with perazine. However, to draw final conclusions about the structural parameters leading to high-affinity binding and strong induced Cotton effects in the case of the interaction of phenothiazine derivatives with α_1-acid glycoprotein, more data using more derivatives are needed.

In conclusion, the data reported indicate that for the phenothiazine neuroleptics investigated in this study, binding to α_1-acid glycoprotein is mediated mainly by only one common high-affinity binding site of the protein.

REFERENCES

(1) K. M. Piafsky, *Clin. Pharmacokinet.*, **5**, 246 (1980).

(2) K. M. Piafsky and O. Borga, *Clin. Pharmacol. Ther.*, **22**, 545 (1977).

(3) M. K. Romach, K. M. Piafsky, J. G. Abel, V. Khouw, and E. M. Sellers, *Ibid.*, **29**, 211 (1981).

(4) S. El-Gamal, U. Wollert, and W. E. Müller, *J. Pharm. Pharmacol.*, **34**, 152 (1982).

(5) J. Schley, M. Siegert, and B. Müller-Oerlinghausen, *Eur. J. Clin. Pharmacol.*, **18**, 501 (1980).

(6) J. Schley, M. Nündel, M. Siegert, E. Riedel, and B. Müller-Oerlinghausen, *Pharmacopsychiatry*, **13**, 144 (1980).

(7) M. Brinkschulte and U. Breyer-Pfaff, *Naunyn-Schmiedeberg Arch. Pharmacol.*, **314**, 61 (1980).

(8) M. L. Kornguth, L. G. Hutchins, and B. S. Eichelman, *Biochem. Pharmacol.*, **30**, 2435 (1981).

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

(10) J. Koch-Weser and E. M. Sellers, *N. Engl. J. Med.*, **294**, 311 and 526 (1976).
(11) W. E. Müller and U. Wollert, *Pharmacology*, **19**, 59 (1979).
(12) U. Kragh-Hansen, *Pharmacol. Rev.*, **33**, 17 (1981).
(13) B. Jirgenssons, *Biochim. Biophys. Acta*, **434**, 58 (1976).
(14) H. Glaser and J. Krieglstein, *Naunyn-Schmiedebergs Arch. Pharmacol.*, **265**, 321 (1970).
(15) A. Hulshoff and J. P. Perrin, *J. Med. Chem.*, **20**, 430 (1977).

ACKNOWLEDGMENTS

This study was supported by a grant of the Deutsche Forschungsgemeinschaft and a fellowship grant of the Alexander von Humboldt-Stiftung.

The excellent technical assistance of A. Stillbauer is gratefully acknowledged.

Complexation of Procainamide with Hydroxide-Containing Compounds

V. DAS GUPTA

Received September 18, 1981, from the *University of Houston, College of Pharmacy, Houston, TX 77030.* Accepted for publication April 2, 1982.

Abstract □ The complexation of procainamide with hydroxide-containing compounds, ethanol, fructose, glucose, glycerin, lactose, maltose, propylene glycol, sorbitol, and sucrose, have been studied. Procainamide formed a complex with glucose, lactose, and maltose, all of which contain a hemiacetal group, whereas fructose and sucrose do not. The percent of complex formed was dependent on the pH of the solution, with an optimum range of ~4–5.2. As with glucose, the percent of complex formed was directly related to the concentration of lactose in the solution. In dry mixtures, procainamide did not form a complex with glucose or lactose. The complex formed with lactose or maltose could be completely reversed by adding hydrochloric acid. A similar observation with glucose was reported earlier. In the optimum pH range, equilibrium was established in ~24 hr, and the process of complexation followed the equation for reversible reactions.

Keyphrases □ Complexation—procainamide with hydroxide-containing compounds □ Procainamide—complexation with hydroxide-containing compounds □ Hydroxide-containing compounds—complexation with procainamide

An earlier report (1) reviewed the literature concerning stability and complexation problems of procainamide in the presence of glucose. The report also presented the results of a study on the complexation of procainamide with glucose. (These two compounds are often mixed in hospitals for intravenous infusion for the treatment of cardiovascular diseases.)

Since procainamide oral dosage forms also are used widely, the formation of complex with some of the excipients seemed possible. The present report investigated the formation of procainamide complexes with hydroxide-containing compounds, ethanol, fructose, glucose, glycerin, lactose, maltose, propylene glycol, sorbitol, and sucrose.

EXPERIMENTAL

Materials—All chemicals and reagents were USP, NF, or ACS grade and were used as received. Procainamide hydrochloride[1] (I) was used without further purification.

A high-performance liquid chromatograph[2], equipped with a multiple wavelength detector[3], a recorder[4] and digital integrator[5], was used.

A semipolar column[6] (30 cm long × 4-mm i.d.) consisting of a monomolecular layer of cyanopropylsilane permanently bonded to silica gel was used.

Chromatographic Conditions—The mobile phase was 40% (v/v) acetonitrile in water containing 0.02 *M* ammonium acetate (pH ~7)[7], and the flow rate was 2.0 ml/min. The detector was set at 280 nm (the wavelength of maximum absorption), the sensitivity was 0.04, the temperature was ambient, and the chart speed was 30.5 cm/hr.

Methods—The stock solutions of procainamide hydrochloride (1.0 mg/ml) and the internal standard, methapyrilene hydrochloride (5.0 mg/ml) in water, were prepared fresh daily. A standard solution was prepared by transferring a 1.5-ml quantity of the stock solution of I and a 4.0-ml quantity of the stock solution of methapyrilene hydrochloride (II) to a 100-ml volumetric flask and then diluting with water to volume.

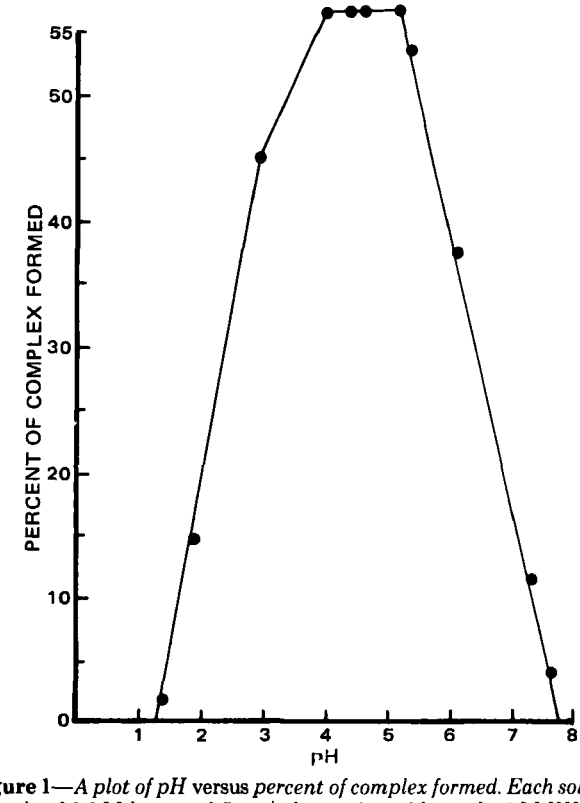


Figure 1—*A plot of pH* versus *percent of complex formed. Each solution contained 0.2* M *lactose, 0.5 mg/ml procainamide, and 0.2* M KH_2PO_4. *Solution of pH 1.4 was buffered with ~1* N *HCl. A similar plot was obtained with glucose.*

[1] Supplied by E. R. Squibb & Sons, Princeton, N.J.
[2] Waters ALC 202 equipped with U6K universal injector, Milford, Mass.
[3] Schoeffel SF770, Westwood, N.J.
[4] Omniscribe 1513-12, Houston Instruments, Austin, Tex.
[5] Autolab minigrator, Spectra Physics, Santa Clara, Calif.
[6] Waters, μBondapak/CN.
[7] Beckman Zeromatic (SS-3) pH meter.

0022-3549/83/0200-0205$01.00/0