product **1b** as a highly hygroscopic solid, nmr (DMSO-d_6) as expected. *Anal.* ($C_7H_{14}NO_5P \cdot XH_2O$) N/P: calcd, 1.00; found, 1.04.

The NH_4 salt of **1b** was prepared by dissolving the phosphate in several milliliters of 2% NH_4OH and freeze drying to a white powder. *Anal.* ($C_7H_{14}NO_5P \cdot 2NH_3 \cdot XH_2O$) N, P: calcd, 3.00; found, 2.98.

***trans*-3-Allophanoylcyclohexyl Diphenyl Phosphate (6d). Method E.**—A solution of 1.0 g (0.0026 mole) of *trans*-3-diphenylphosphorylcyclohexanecarboxylic acid (**6a**) in 25 ml of anhydrous C_6H_6 was treated with 0.4 g (0.0032 mole) of oxalyl chloride at 25°. After the initial effervescence had subsided the reaction mixture was stirred overnight at 25°, heated for 1 hr at 60°, and evaporated *in vacuo* to give a syrup. The syrup was then treated with 0.50 g (0.008 mole) of urea at 65° for 2 days. The resulting brown mass was partitioned between EtOAc and H_2O and the EtOAc extracts were dried ($MgSO_4$). Subsequent filtration and evaporation afforded 1.0 g (90%) of the desired product (**6d**) as an impure brown oil. Purification was accomplished on silica using 2% MeOH–$CHCl_3$ as the eluent; ir (liquid film) and nmr as expected. *Anal.* Calcd ($C_{20}H_{23}N_2O_6P$) C, H, N, P.

Trifluoroacetylurea.—Urea (26 g, 0.44 mole) was dissolved in 100 ml of CF_3COOH, and 93 g (0.44 mole) of $(CF_3CO)_2O$ and 0.1 ml of H_2SO_4 were added. The solution was heated to 100° for 1 and stirred at 25° for 2 hr, and 500 ml of H_2O was added. The solid product was collected and recrystallized from EtOH; mp 184–186° (lit.[14] 189°). *Anal.* ($C_3H_3F_3N_2O_2$) C, H, N.

Cyclohexylcarboxylurea.—Cyclohexylcarboxylic acid (2 g, 16 mmoles) was stirred with 15 ml of $SOCl_2$ overnight. The solution was evaporated to a thick syrup and heated to 70° for 1 day with 2 g (33 mmoles) of urea. Upon the addition of 30 ml of H_2O a solid formed which was filtered and dried to yield 2.26 g of the product (83%) which was recrystallized from EtOH; mp 230–232°. *Anal.* ($C_8H_{14}N_2O_2$) C, H, N.

Acknowledgments. The authors thank Professor Robert A. Wiley for assistance during the absence of M. P. M., Mrs. Wen Ho and Mrs. Richard Wiersema for the enzyme inhibition studies, and Dr. Mohammed Shipchandler and Miss Cindy Hartzell for the synthesis of cyclohexyl phosphate and cyclohexylcarboxylurea.

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A Physicochemical Model for the Mechanism of Action of Antihistaminics and Cortisol

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Received March 19, 1969

The mechanism of action of antihistaminics and cortisol has been studied at the molecular level using "coupled" ion-exchange membrane electrodes on an *in vitro* system consisting of bovine serum albumin as the receptor and various antihistaminics and cortisol as competitors. The data obtained indicate that, when the antihistaminic concentration is above a critical value (1×10^{-4} *M*), histamine does not induce those changes in bovine serum albumin which are necessary for an interaction. At the same time, structural analogs without antihistaminic activities have shown no influence on the binding of histamine, when tested in the same experimental conditions. Furthermore, the access of histamine to the biopolymer is inhibited by molar concentrations of antihistaminics which closely approximate those previously found for inorganic monovalent ions. The correlation between ionic strength and antihistaminic action is discussed in terms of a stabilizing effect of the antihistaminic on a given conformation of the biopolymer. This conformation is unable to bind histamine. When antihistaminics are replaced by cortisol, this steroid prevents the binding of histamine to the macroion at molarities at which antihistaminics show no effect. In fact, under the same experimental conditions, cortisol is more effective by a factor of 10 than antihistaminics in stabilizing that conformation of the biopolymer which does not interact with histamine.

The investigation of pharmacological problems has been greatly aided by the study of model systems using a purely physicochemical approach. This is particularly true for the mechanism of action of antihistaminics. Many of the mechanisms postulated[1] may be tested at the molecular level using a synthetic *in vitro* system. Even though the model system is merely a method for visualizing a problem in simpler molecular terms and is not an attempt to reproduce physiological conditions, it can serve the purpose of eliminating those mechanisms which violate the principles obtained from these studies.

Kier's considerations[2] on the interatomic distances in the cortisol and histamine molecules and theoretical speculations on a possible competition between the two as an explanation of the role of cortisol in controlling the inflammatory response have found experimental support in the electrochemical data presented in this paper. We have demonstrated that an electrostatic competition between cortisol and histamine does occur and no binding of histamine to the macroion takes place in the presence of a given molar concentration of cortisol.

It is commonly believed[1] that the antihistaminics function by competing with histamine for a specific receptor site on a protein. This receptor is ill defined and has not, as yet, been isolated or identified.

On the assumption[3] that protein–drug interaction produces a change in the structure of the biopolymer and consequently a variation in the mean ionic activity of the saline medium, potentiometric measurements have been carried out by means of "coupled" ion-exchange membrane electrodes previously described.[4] This new technique is useful for the study of unstable biologically important compounds and of biopolymers which undergo conformational changes.[5]

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(3) (a) E. J. Ariens, *Mol. Pharmacol.*, **1**, 246 (1964); (b) *Advan. Drug Res.*, **3**, 446 (1966).
(4) C. Botré, S. Borghi, and M. Marchetti, *Biochim. Biophys. Acta*, **135**, 208 (1967).
(5) (a) C. Botré, S. Borghi, M. Marchetti, and M. Baumann, *Biopolymers*, **4**, 1046 (1966); (b) *ibid.*, **5**, 483 (1967); (c) C. Botré', M. Marchetti, and S. Borghi, *Biochim. Biophys. Acta*, **154**, 360 (1968); (d) L. Bolis, C. Botré, S. Borghi, and M. Marchetti in "Membrane Models and the Formation of Biological Membranes," L. Bolis and B. A. Pethica, Ed., North-Holland Publishing Co., Amsterdam, 1968; (e) C. Botré, *Farmaco, Ed., Sci.*, **5**, 411 (1968).

This technique permits us to examine the molecular events which occur during histamine binding to bovine serum albumin. In several antihistaminics of different chemical composition the antihistaminic activity ran parallel to the effect of the ionic strength[5c] on the structural stability of bovine serum albumin. It has been previously demonstrated[5c,e] that the bovine serum albumin–histamine interaction is dependent on ionic strength, that is, there is a critical saline concentration which can be regarded as a threshold defining two sharply different behaviors of bovine serum albumin in the presence of histamine. It was suggested that bovine serum albumin undergoes structural changes which seem to be triggered by histamine. These structural changes are regulated by the ionic strength of the medium which hinders the attachment of histamine to the macromolecule when a critical salt concentration is reached.

Since an increase in the total ionic strength of the medium will reduce the electrostatic interaction between opposite charges,[6] this limiting influence could not account for either the structural change or the threshold significance of the effect.

Experimental Section

Materials.—Crystallized bovine serum albumin obtained from British Drug Houses was dialyzed against water for 5 days at 4° and then lyophilized.

Diphenhydramine hydrochloride, promethazine hydrochloride, clemizole hydrochloride, methapyrilene hydrochloride, and Antergan were further crystallized before use. Hydrocortisone-21 sodium succinate was the generous gift of the Upjohn Co., Milan, Italy. All other reagents employed were of analytical grade.

Membranes.—The membranes used in this work were made of sulfonated polystyrene embedded in a collodion matrix. The preparation and the use of such membranes as electrodes have been discussed extensively elsewhere.[4]

Two types of membranes M_1 and M_2 with different charge densities of ionizable groups were prepared, *i.e.*, M_1 was 5×10^{-4} equiv/kg and M_2 was 5×10^{-1} equiv/kg. The cell can be schematized as follows

standard calomel electrode	reference saline solution		M_1	bovine serum albumin	M_2	reference saline solution	standard calomel electrode

where the reference saline solution had a concentration of 0.01 M.

Procedure.—In the central compartment of the cell, a solution of the antihistaminics (1×10^{-4} M) containing bovine serum albumin (1 mg/ml) was titrated with a concentrated solution of histamine (0.1 M). In a blank titration the histamine solution was replaced with distilled H_2O in order to evaluate the contribution to the electromotive force made by the dilution of bovine serum albumin. All potentiometric measurements were performed at room temperature with constant stirring. A Radiometer pH meter M_4 apparatus was used.

Conductivity measurements were made with a WTW model LF_3 bridge in a thermostated bath (25 ± 0.02°).

Dialysis was carried out with cellophane bags, HMC type, previously treated with boiling H_2O for about 5 min, washed (cold H_2O), wiped, and filled with a given volume of histamine solution having the same concentration as the one contained inside the bag. The equilibrium during dialysis was reached at room temperature in about 20 hr. No significant changes in volume inside or outside the bag were observed. A grating spectrophotometer Hitachi-Perkin-Elmer Model 139 equipped with quartz cells covering the optical path from 10 to 0.2 mm was used. At each concentration a blank test was set up. In this case H_2O replaced the protein solution inside the bag.

In all measurements, the bovine serum albumin concentration was constant (1 mg/ml), while the histamine concentration varied. The pH values were between 4.2 and 4.7 and no shift from these values was observed throughout the measurements.

(6) P. Debye and E. Huckel, *Physik. Z.*, **24**, 185 (1923).

Results and Discussion

Specific conductivity measurements of histamine solutions at different concentrations have shown that no aggregation takes place in the range of concentration used in these studies. At the same time no discontinuity can be detected by specific conductivity of a bovine serum albumin solution (in NaCl 1×10^{-4} M) of increasing concentration.

In Figure 1, specific conductivity, χ, is plotted as a function of histamine molarity in a solution of bovine serum albumin whose concentration is kept constant (1 mg/ml) and contains differing amounts of antihistaminics.

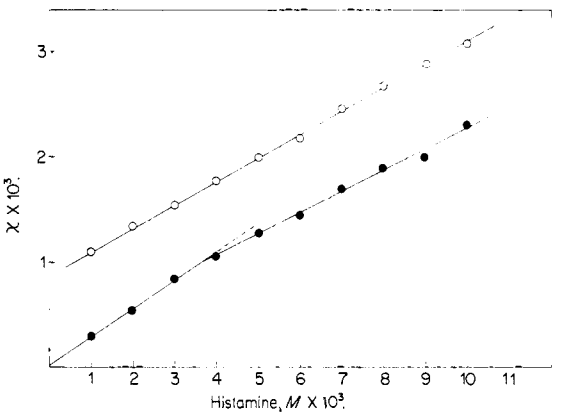

Figure 1.—Specific conductivity, χ, *vs.* histamine molarity in a solution of bovine serum albumin (1 mg/ml) containing diphenhydramine hydrochloride (○–○, $1 \times 10^{-2}M$; ●–●, $1 \times 10^{-4}M$).

The plots refer to two different experimental conditions which are characterized by the concentration, 1×10^{-2} and 1×10^{-4} M, respectively, of the antihistaminics present in the medium and whose value is kept unaltered throughout the measurements.

The transition point in one of the two plots of this figure marks the "complex" formation and gives the value for the ratio by weight of histamine to bovine serum albumin in the complex. At the same time no discontinuity and therefore no binding can be detected in the other plot. Thus an increase in the concentration of the antihistaminic present inhibits the binding of histamine to the macroion in the range of concentrations studied.

Similar results (Figure 2) were obtained in investigating the bovine serum albumin–histamine–antihistaminics system by means of membrane equilibrium dialysis according to a procedure proposed by Klotz and Walther.[7] Here the diffusion of histamine occurs at two different rates. When the antihistaminic concentration is above the critical value of 1×10^{-4} M the system behaves as if the nondiffusible macromolecule was not present and histamine diffuses through the dialysis membrane according to its concentration gradient.

By contrast, when the antihistaminic concentration is equal to 1×10^{-4} M, the histamine added is "captured" by the biopolymer. Therefore membrane equilibrium dialysis further substantiates the observed effects which occur for concentration values of the constituents approximating those found through conductivity measurements.

(7) J. M. Klotz and F. M. Walther, *J. Am. Chem. Soc.*, **70**, 943 (1948).

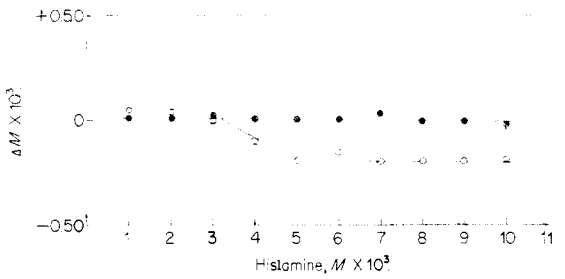

Figure 2.—Membrane equilibrium dialysis proves the existence of a competition between histamine and antihistaminic cations. ΔM is plotted as a function of the original histamine molarity, when antihistaminics (diphenhydramine hydrochloride) are present at two different concentrations (●–●, 1×10^{-3} *M*; O–O, 1×10^{-4} *M*). ΔM is the variation of histamine concentration in the outside compartment after equilibrium is reached. Changes in histamine concentration in the outside compartment are determined by means of ultraviolet absorption at λ 212 mμ. The equilibrium is reached within 20 hr. The reading is done after 30 hr.

In Figures 3 and 4 the electromotive force (in mV) recorded in solutions of bovine serum albumin (1 mg/ml) containing different antihistaminics at the same concentration (1×10^{-4} *M*) is plotted *vs.* the logarithm of histamine molarity. The data obtained show the same general shape and break points regardless of which antihistaminic is present. On the other hand, when the antihistaminic concentration is above the critical value (1×10^{-4} *M*), histamine does not induce those changes in bovine serum albumin which are necessary for interaction. Consequently, the resulting plots are straight lines and no transition point can be detected.

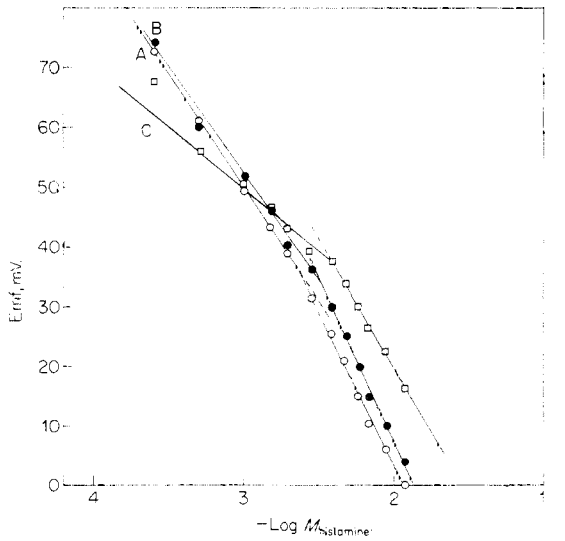

Figure 3. Electromotive force (in mV) *vs.* logarithm of histamine concentration in a solution of bovine serum albumin (1 mg/ml) containing different antihistaminics at the same molar concentration (1×10^{-4} *M*). Plot A, O–O, diphenhydramine hydrochloride; plot B, ●–●, methapyrilene hydrochloride; plot C, [illegible], clemizole hydrochloride.

It is well known that the binding of an organic or inorganic cation to a polypeptide in solution will stabilize that conformation of the many in equilibrium with each other and approximately of equal energy, which permits the interaction of appropriate groups of the protein with the various portions of this organic

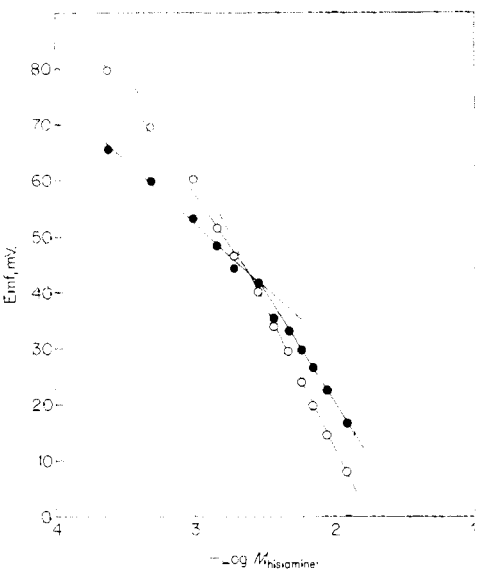

Figure 4.—Electromotive force (in mV) *vs.* logarithm of histamine concentration in a solution of bovine serum albumin (1 mg/ml) containing different antihistaminics at the same molar concentration (1×10^{-4} *M*). Plot A, O–O, promethazine hydrochloride; plot B, ●–●, Antergan.

cation.[8] If the effect of a drug depends, among other factors, on the capacity of the drug molecule to induce changes in the conformation of the biopolymer which are required for the induction of the stimulus and consequently the effect of the drug,[9] then the correlation between ionic strength and antihistaminic action can be discussed in terms of a stabilizing effect of the antihistaminic on a given conformation of the biopolymer. This conformation is unable to bind histamine.

It may then be assumed that the binding sites on the biopolymer are the same for both alkali ions[9b,c] and antihistaminic cations, while this does not apply to the forces which are responsible for the binding itself.

To gain an insight into this problem we substituted the antihistaminics with other organic cations analogous to them, but without their pharmacological activity. For this purpose we resorted to several local anesthetics which were incapable of inhibiting the access of histamine to the macroion when added in the same concentration as the antihistaminics previously used.

The results are presented in Figures 5 and 6 where specific conductivity and potentiometric measurements show that histamine is bound to the macroion in the presence of a local anesthetic (procaine hydrochloride) at a concentration of 1×10^{-2} *M*.

It is well known that cortisol and its 17-OH derivatives[9] exert a potent antiinflammatory action. From a calculated relationship between interatomic distances in cortisol and histamine molecules it has recently been suggested[1] that cortisol might function as an antagonist of histamine in the binding to the receptor. This would imply that cortisol produces its pharmacological effect by competing with histamine by a mechanism such as agonist–antagonist receptor interaction.

In Figure 7, the specific conductivity, χ, is plotted as a function of histamine molarity in a solution of bovine

(8) F. Karush, *J. Am. Chem. Soc.*, **72**, 2705 (1950).

(9) (a) W. W. Swingle, E. J. Fehr, R. A. Maxwell, M. Bell, and G. Barlow, *Am. J. Physiol.*, **172**, 527 (1953); (b) R. W. Schayer, K. J. Davis, and R. L. Smiley, *ibid.*, **182**, 54 (1955); (c) R. W. Schayer, *Ann. N. Y. Acad. Sci.*, **116**, 891 (1964).

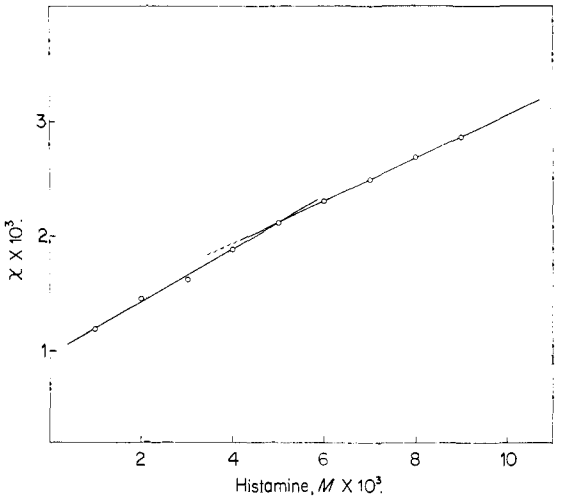

Figure 5.—Specific conductivity, χ, *vs.* histamine molarity in a solution of bovine serum albumin (1 mg/ml) containing procaine hydrochloride (1×10^{-2} *M*).

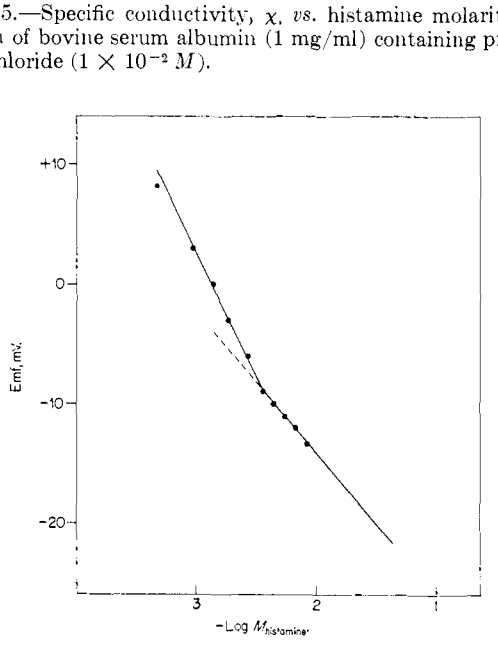

Figure 6.—Electromotive force (in mV) *vs.* logarithm of histamine concentration in a solution of bovine serum albumin (1 mg/ml) containing procaine hydrochloride (1×10^{-2} *M*).

serum albumin whose concentration is kept constant (1 mg/ml) in the presence of hydrocortisone-21 sodium succinate at two different values of concentration (1×10^{-4} and 1×10^{-5} *M*). In Figure 8, the electromotive force (in mV) recorded in a solution of bovine serum albumin (1 mg/ml) containing different amounts (1×10^{-4} and 1×10^{-5} *M*) of hydrocortisone-21 sodium succinate is plotted *vs.* the logarithm of histamine molarity. In both these cases the electrochemical data indicate that competition exists between cortisol and histamine and that a well-defined concentration of cortisol inhibits the binding of histamine to the macroion.

By comparing Figures 3 and 4, one finds that the values of the recorded electromotive force and the slope of the first part of the plots show that at this stage the antihistaminics are being "dislocated" from the bovine serum albumin while histamine takes their place. At the same time the shift in pH (from 4.7 to 4.2) contributes to the increase in ionic activity as indicated by the decrease in the recorded electromotive force. The slope of the second part of the plots shows

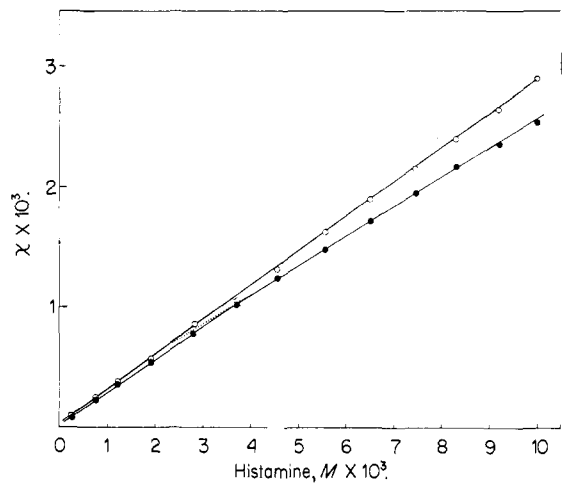

Figure 7.—Specific conductivity, χ, *vs.* histamine molarity in a solution of bovine serum albumin (1 mg/ml) in the presence of hydrocortisone-21 sodium succinate at two different molar concentrations (O–O, 1×10^{-4} *M*; ●–●, 1×10^{-5} *M*).

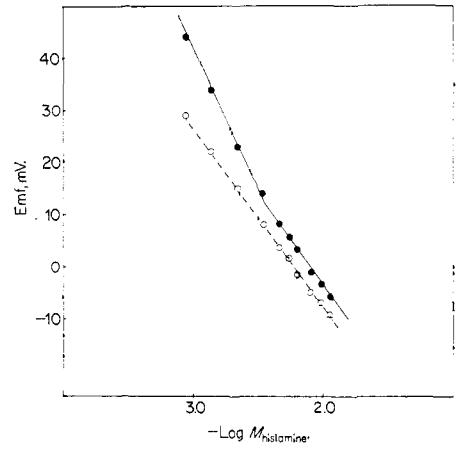

Figure 8.—Electromotive force (in mV) *vs.* logarithm of histamine molarity in a solution of bovine serum albumin (1 mg/ml) containing different amounts of hydrocortisone-21 sodium succinate (O–O, 1×10^{-4} *M*; ●–●, 1×10^{-5} *M*).

that, after the transition point, any further addition of histamine will influence the electromotive force as though the macromolecular "complex" was not present. The slope of this part of the curve is almost coincident to that of a solution of histamine in the same range of concentration.

The situation is quite different in Figure 6 where the slope of the first part of the plot approximates that of a solution containing just histamine and/or local anesthetic, while the second part shows the binding of histamine to the macroion.[5c]

The shift in potential values observed when going from antihistaminics to local anesthetics, is due to solutions of the same ionic strength (1×10^{-2} *M*) being contained in the three compartments of the cell. The effect of cortisol on the macroion seems to be more extensive than that produced by antihistaminics. In fact, under the same experimental conditions, cortisol is more effective by a factor of 10 than the antihistaminics in preventing the binding of histamine. It seems reasonable to assume that the binding of cortisol to the macroion renders its structure less susceptible to the action of histamine.

The technique and the principles so far illustrated

can find successful use in the approach to many pharmacological problems which involve competitive mechanisms. It will still remain open to question how far the chosen protein can be compared to the physiological receptor. Nevertheless, so little is known about physiological receptors that their protein nature is the only positive fact on this subject to date that can be put to use in hopes of gaining some insight on how to proceed in the infinitely more complex physiological system.

Bicyclic Homologs of Piperazine. IX.[1,2] Synthesis and Pharmacological Properties of Phenothiazine and of 10,11-Dihydrodibenzocycloheptene Derivatives of 3,8-Diazabicyclo[3.2.1]octanes

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Received February 10, 1969

All the psychotropic phenothiazines contain a basic substituent connected to the N of the tricyclic system, through a $(CH_2)_3$ chain. Since the basic group of many representative phenothiazines is a piperazine derivative,[3] we introduced, instead of this nucleus, that of 3,8-diazabicyclo[3.2.1]octane, in which a piperazine ring is embodied. The two nonequivalent basic nitrogens of 3,8-diazabicyclo[3.2.1]octane allow the synthesis of isomeric compounds; in fact, the three-carbon side chain may be attached to N-8 or to N-3. We have also synthesized two 10,11-dihydrodibenzocycloheptene derivatives of 3,8-diazabicyclo[3.2.1]octane, structurally related to the drug amitriptyline.[4] The psychotropic activity of the new compounds was evaluated.

Chemistry.—The phenothiazines synthesized (I-1–6, II-1–4) are summarized in Table I. The 10,11-dihydrobenzocycloheptene derivatives (III, IV) are reported in the Experimental Section. As indicated in Scheme I, the synthesis of I and II was carried out by condensing the appropriate 10-(γ-chloropropyl)phenothiazines[4,5] (V) with 3- or 8-substituted 3,8-diazabicyclo[3.2.1]-octanes (VI–IX)[6,7] in the presence of powdered NaOH in refluxing PhMe (method A), or NEt_3 in refluxing AcPr (method B). Alternatively I-1 and II-1 were synthesized by condensing VI and VIII with 1-bromo-3-chloropropane to give the corresponding γ-chloropropyl derivatives X and XI which were allowed to react with 2-chlorophenothiazine (method C). Compound VII was obtained by addition of ethylene oxide to 8-propionyl-3,8-diazabicyclo[3.2.1]octane[8] followed by the removal of the 8-propionyl group by acid hydrolysis. The isomer IX was synthesized either by addition of ethylene oxide to 3-benzyl-3,8-diazabicyclo-[3.2.1]octane,[9,10] followed by catalytic debenzylation of the intermediate, or by addition of ethylene oxide to 3,8-diazabicyclo[3.2.1]octane.[9]

Finally, III and the isomer IV were synthesized by treating dibenzosuberone with the Grignard reagent of X and XI to give the tertiary alcohols XIV and XV, which were eventually dehydrated with *p*-toluenesulfonic acid in refluxing toluene.

Results and Discussion

The results of pharmacological studies are briefly summarized in Table II. Many 3,8-diazabicyclo[3.2.1]-octane derivatives show an effect on animal behavior (decrease of spontaneous motor activity and inhibition of conditioned response) in doses relatively low, even compared with chlorpromazine. Moreover, no compound produces side effects, such as α-adrenergic blocking and antihistaminic action, which are seen with chlorpromazine and other phenothiazine derivatives, to a higher extent than chloropromazine itself. Particularly good results were obtained with 3-(β-hydroxyethyl)-8-(2-trifluoromethyl-10-phenothiazinylpropyl)-3,8-diazabicyclo[3.2.1]octane (I-6), which is three to six times more active than chlorpromazine on behavior, and equally or less active in blocking the pressor response to epinephrine and antagonizing the action of histamine. The activity of I-6 seems worthy of further evaluation in view of a practical interest in the compound as a possible major tranquilizer. Relationships between structure and activity suggest that better results are obtained when the three-carbon side chain of phenothiazine is attached to N-8 of 3,8-diazabicyclo-[3.2.1]octanes instead of N-3. The β-hydroxyethyl group on N-3 of 3,8-diazabicyclo[3.2.1]octane nucleus is also more favorable for tranquilizing activity than Me. As in other phenothiazine derivatives, activity increases when substitutions are made in the 2 position of the phenothiazine ring with Cl or F_3C. Although the results need a more extended analysis, it may be pointed out that, generally, the basic side chain of psychotropic phenothiazine derivatives may be substituted with 3,8-diazabicyclo[3.2.1]octane at N-8, without substantial decrease of the pharmacological effectiveness and, perhaps with an advantage, at least in some cases, in the specificity of action. The 3,8-diazabicyclo[3.2.1]octane derivatives, structurally related to amitriptyline (III and IV), showed little or no

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