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SOME CHEMICAL AND PHYSICAL PROPERTIES ASSOCIATED WITH HISTAMINE ANTAGONISM

BY

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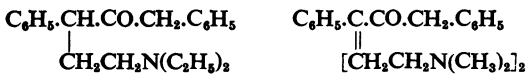
To study the relationships between chemical structure, physical properties and antihistamine activity, it is necessary to measure the relative potencies accurately, and to determine which compounds are competitive antagonists of histamine and which are merely non-competitive spasmolytics. The *p*A technique of Schild (1947) provides a method whereby both these objects may be achieved, and a relationship between antihistamine action and dissociation constant previously observed has been confirmed.

METHODS

The following series of compounds have been studied :

(1) The " Wilson " series, whose chemical structures have been recorded by Marshall, Ahmad, and Weston (1952). The numbers previously assigned to the compounds are also used in the present paper. Two further compounds, both oily bases, have been numbered 20A and 20B ; their structures are :

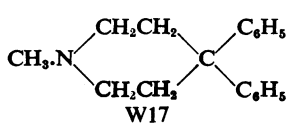


20A

20B

Potentiometric titration of 20B showed it to be chemically unstable, and it was not therefore included in the pharmacological investigation.

(2a) Methadone, and a series of related compounds which have been described by Walton, Ofner, and Thorp (1949) and by Ofner and Walton (1950) ; these are referred to in Table II by code numbers. Two compounds not referred to in the literature have the structures :



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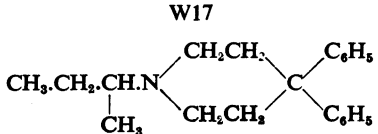

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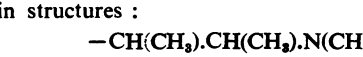
(2b) Phenadoxone, *iso*-phenadoxone, and five related compounds which are referred to in Table II by figures denoting the number of the compound in Table II of the paper by Dupré, Elks, Hems, Speyer, and Evans (1949).

(3) A series of proprietary antihistamine drugs.

(4) A series of phenothiazine derivatives. Structures of all but three of these have been described by Viaud (1954) ; these three have the following side-chain structures :



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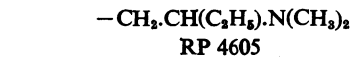

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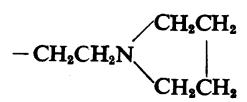

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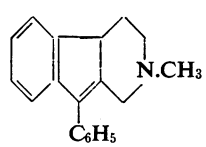
(5) A series of benzhydryl derivatives allied to chlorcyclizine (Table I).

(6) Two derivatives of phenindamine :



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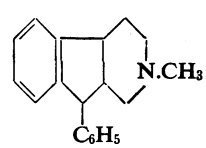


Nu-1525

Measurement of Antihistamine Action

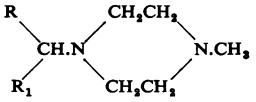
The *p*A values were determined by the method of Schild (1947) using an automatic organ bath in which guinea-pig ileum was suspended in oxygenated Tyrode solution at 36° C. Doses of histamine were added every 2 min. and contact with each concentration of antagonist was maintained for five cycles. The basic concentration of histamine was 0.02 μg./ml. Each pA_2 and pA_{10} value is the mean of the results on ileum from at least four guinea-pigs.

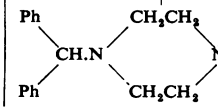
At first the *p*A values for the more potent antihistamines were discrepant because the basic compounds were adsorbed on to the glassware. This source of error has been described for botulinus

TABLE I

STRUCTURES OF BENZHYDRYL (CHLORCYCLIZINE) SERIES

General formula



Serial No.	R	R_1	Salt
A-1198 ..	Phenyl	Phenyl	HCl
A-2285 ..	*p*-Bromo-phenyl	,,	,,
A-2342 ..	*p*-Fluoro-phenyl	,,	,,
A-2688 ..	*p*-Methyl-phenyl	,,	,,
Chlorcyclizine	*p*-Chloro-phenyl	,,	,,
A-2824 ..	*m*-Chloro-phenyl	,,	,,
A-2917 ..	2-Thiophen	*p*-Chloro-phenyl	$2(COOH)_2$
A-4826 ..			,,

toxin by Bronfenbrenner and Schlesinger (1922), and for bases by Brodie, Udenfriend, and Baer (1947). Tests by experimental contamination of flasks and pipettes with chlorothen showed that adsorption on to glass surfaces could be eliminated by soaking the glassware in commercial conc. HNO_3 when not in use (to avoid the formation of surface films of substances such as detergents), and by using a minimum number of stages in preparing the high dilutions required for *p*A determinations.

Measurement of Dissociation Constants

Dissociation constants were calculated from data obtained by titrating fractional molar solutions of salts of basic compounds with standard KOH. Because of the small amounts of some compounds, 20 ml. portions of 0.0025M solutions were titrated with an equivalent volume of 0.1N-KOH. With monobasic compounds, 0.5 ml. 0.1N-KOH was added in ten 0.05 ml. portions, using an Agla micrometer syringe, clamped horizontally, and fitted with a glass attachment bent at right angles, with the fine tip 0.5 mm. above the surface of the solution. With dibasic compounds, 1.0 ml. 0.1N-KOH was required to complete the titration. After each addition, the *p*H of the solution was recorded by means of standard glass and calomel electrodes connected to a Cambridge *p*H meter. The titrations were carried out at 20° C. All solutions were prepared with distilled water boiled for 20 min. and stored in an aspirator fitted with a soda-lime absorption tube. The 0.1N-KOH was prepared from washed sticks of potassium hydroxide AR dissolved in boiled water. After standardization, the solution was adjusted to volume and stored in an aspirator of amber glass fitted with a soda-lime tube. The solution was re-standardized every 14 days, using the *p*H meter, the titration curve being checked against the theoretical curve in order to detect any conversion of hydroxide to carbonate (Fig. 1).

Where, as usually, the base was available as a salt of a strong acid such as sulphuric or hydrochloric, the salt was dissolved and titrated as such. Compounds supplied as the free base were dissolved in the stoichiometric amount of HCl before making up to volume with boiled water. Where the compound was supplied as the salt of a weak organic acid, such as citrate, maleate or phosphate, the weak acid affected the titration curve. Here it was necessary to convert the compound to a salt of a strong acid. Stoichiometric portions of the weak acid salt (usually sufficient to make 50 or 100 ml. of 0.0025M) were dissolved in water in a separating funnel, and the exact equivalent of 0.1N-KOH added to liberate the free base. The free base was transferred to ethereal solution by shaking with two successive 30 ml. portions of ether. The mixed ether extract was then re-extracted with an exact equivalent of 0.1N-HCl, in three successive portions. The mixed acid extract was heated on a water bath to drive out dissolved ether, and made up to volume with boiled water. Aliquots of 20 ml. of the hydrochloride were then titrated with 0.1N-KOH.

Where the free base precipitated during titration, it was necessary to carry out the titration in alcoholic solution. In this wide range of compounds it was not practicable to apply a constant alcohol correction of +0.5 *p*H unit as used by Albert, Rubbo, Goldacre, Davey. and Stone (1945) for a series of acridines, since the slopes of the pK_a – % alcohol curves differed for

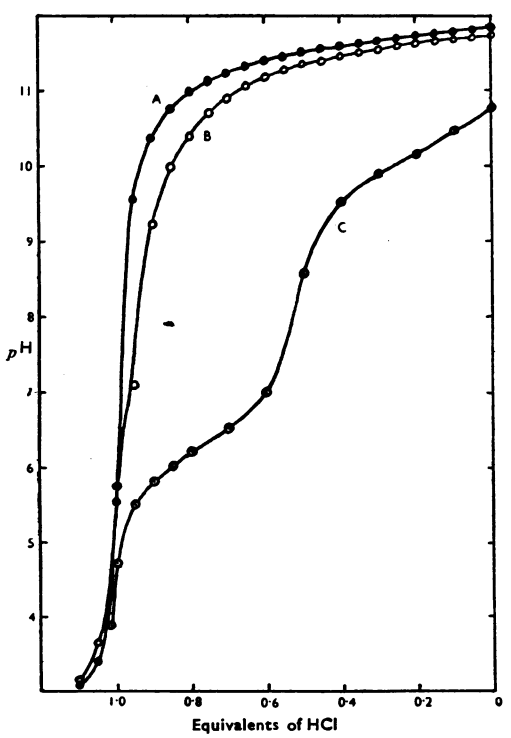


FIG. 1.—The titrimetric standardization of potassium hydroxide solution. The effect of carbonate contamination on the titration curve. A=pure KOH. B=KOH, 90%; Na_2CO_3, 10%. C=pure Na_2CO_3.

different groups of compounds (total range 0.20 to 0.545). The method described by Mizutani (1925) was therefore adopted, each compound being titrated in three different concentrations of alcohol (usually 30, 40, and 50%) and the values extrapolated back to zero % alcohol.

The dissociation constant (pK_a) was calculated from each pH reading on the titration curve according to the formula :

$$pK_a = pH - \log \frac{[B]}{[BH^+]}$$

the values for $[BH^+]$, the concentration of unchanged salt, and [B], the concentration of liberated free base, being derived from the titration figure. Where the pH readings were high, it was necessary to apply a correction for the concentration of free OH^- ions in solution, when the formula becomes :

$$pK_a = pH - \log \frac{[B] - [OH^-]}{[BH^+] + [OH^-]}$$

the values for $[OH^-]$ being derived from the observed pH. It is usual to apply this correction to all pH readings above 10, but, because of the low concentrations titrated, it was necessary to apply the correction to all readings above pH 9.

Determinations of pK_a values carried out on different samples of the same compound—in some cases at intervals of over two years—showed good agreement. For example, pK_a measurements on four samples of RP 3277 between 1950 and 1952 showed a range of 0.16 pK unit, and duplicate determinations on six other compounds differed by an average of 0.07 pK unit.

Results

Table II records the values for pK_a, pA_2, and pA_{10} for the compounds studied. Standard deviations of the values for pA_2 and pA_{10} are shown in parentheses. In a few of the "Wilson" series, technical difficulties prevented determinations of either pK_a or pA values. With compound 15, almost complete insolubility precluded either physical or pharmacological determinations, whereas with compound 13 the low pharmacological activity and relatively low solubility made it impossible to determine pA_{10}. With compounds A-2917 and A-4826 of the benzhydryl series (Table II) it was not possible to obtain characteristic titration curves even after conversion of the oxalate to hydrochloride. This might be expected from the structure of A-4826 (Table I), but it is hard to see why A-2917 should not show normal basic properties.

Distinction Between Competitive and Non-competitive Antagonists

Competitive and non-competitive antagonists may be distinguished by the difference between pA_2 and pA_{10}. When pA_{10} is measured, nine times as many molecules of histamine are being antagonized as when pA_2 is determined. If the antagonist functions by competing with histamine, it will require nine times as many molecules to antagonize a ninefold increase in histamine molecules, and the difference between pA_2 and pA_{10} will be ninefold, or 0.95 on the pA scale. If the antagonist is non-competitive, inhibiting only the contraction mechanism of the muscle, a smaller

TABLE II

pK_a AND pA_x VALUES

S.D. in parentheses. Type of antagonism: +, competitive; −, non-competitive; ⊕, probably competitive (see text)

Serial No. or Name	pK_a	pA_2	pA_{10}	(pA_2-pA_{10})	Type of Antagonism
"Wilson" Series					
1	8·37	3·91 (0·05)	3·25 (0·14)	0·66	−
2	9·04	4·06 (0·02)	3·56 (0·12)	0·50	−
3	8·30	4·70 (0·03)	4·22 (0·07)	0·48	−
4	9·40	4·91 (0·08)	4·43 (0·04)	0·48	−
5	8·80	4·65 (0·12)	3·88 (0·11)	0 77	+
6	9·03	5·13 (0·08)	4·56 (0·09)	0·57	−
7	6·23	3·33 (0·23)	2·75 (0·08)	0·58	−
8	6·17	4·24 (0·14)	3·78 (0·02)	0·46	−
9	8·71	5·46 (0·09)	4·65 (0·07)	0·81	+
10	9·55	5·25 (0·09)	4·64 (0·09)	0·61	−
11	9·45	5·39 (0·07)	4·74 (0·12)	0·65	−
12	6·39	4·50 (0·13)	3·96 (0·09)	0·54	−
13	10·25	3·19 (0·23)	Solubility exceeded		
14	9·38	5·15 (0·15)	4·55 (0·04)	0·60	−
15	Insoluble				
16	9·22	4·48 (0·22)	3·95 (0·09)	0·53	−
17	Ppt. insoluble in alcohol on titration				
18	9·07	5·33 (0·10)	4·09 (0·17)	1·24	⊕
19	8·99	5·26 (0·04)	4·34 (0·10)	0·92	+
20	8·90	5·04 (0·12)	4·57 (0·18)	0·47	−
20A	9·46	5·56 (0·08)	4·58 (0·11)	0·98	+
20B	Unstable				
21	8·99 7·13	5·09 (0·17)	4·45 (0·04)	0·64	−
22	9·68 7·18	4·86 (0·08)	4·32 (0·17)	0·54	−
23	9·99 7·98	5·53 (0·11)	4·52 (0·06)	1·01	+
24	10·09 8·41	4·63 (0·05)	3·82 (0·14)	0·81	+
25	9·78	4·44 (0 07)	4·04 (0 02)	0·40	−
Methadone Series					
50/XII/CN	9·08	5·73 (0·11)	4·73 (0·12)	1·00	+
49/III/CN(+)	8·68	5·44 (0·05)	4·84 (0·06)	0·60	−
(−)	8·67	6·27 (0·23)	5·10 (0·10)	1·17	+
49/IV/CN	8·16	5·14 (0·08)	4·50 (0·10)	0·64	−
50/II/CN	8·93	6·12 (0·08)	4·86 (0·11)	1·26	⊕
50/I/CN	8·83	5·31 (0·11)	4·24 (0·15)	1·07	+
50/XII/COOH	10·59	3·14 (0·22)	2·43 (0·04)	0·71	+
49/III/COOH	10·88	3·64 (0·05)	2·67 (0·03)	0·97	+
49/III/COOEt	10·12	5 41 (0·18)	5·02 (0·19)	0·39	−
50/XII/COOEt	11·59	5·00 (0·16)	4·04 (0·08)	0·96	+
49/V/COOEt	9·87	5·52 (0·05)	4·93 (0·16)	0·59	−
49/III/COBu	10·38	5·65 (0·19)	5·24 (0·21)	0·41	−
49/IV/COMe	9·53	5·01 (0·13)	4·76 (0·14)	0·25	−
50/II/COEt	10·35	5·68 (0·06)	5·09 (0·08)	0·59	−
50/I/COEt	9·40	5·65 (0·11)	4·75 (0·22)	0·90	+
W17	8·65	3·25 (0·13)	2·51 (0·01)	0·74	−
W18	9·67	6·04 (0·20)	5·15 (0·18)	0·89	+
Methadone	10·12	5·78 (0·20)	4·84 (0·18)	0·94	+
17	6·96	4·60 (0·03)	4·28 (0·15)	0·32	−
Phenadoxone	7·70	5·07 (0·20)	4·62 (0·22)	0·45	−
*iso*Phenadoxone	7·12	4·72 (0·29)	4·27 (0·10)	0·45	−
16	6·83	4·52 (0·10)	4·00 (0·11)	0·52	−
2	8·96	5·59 (0·05)	4·97 (0·14)	0·62	−
18	7·17	4·67 (0·11)	4·51 (0·09)	0·16	−
4	9·47	5·79 (0·07)	5·24 (0·09)	0·55	−

TABLE II—*continued*

Serial No. or Name	pK_B	pA_2	pA_{10}	(pA_2-pA_{10})	Type of Antagonism
Proprietary Antihistamines					
Benadryl (diphenhydramine)	8·98	8·14 (0·13)	6·78 (0·19)	1·36	⊕
Pyribenzamine (tripelennamine)	8·95	9·00 (0·24)	7·64 (0·13)	1·36	⊕
Anthisan (mepyramine)	8·85	9·36 (0·26)	8·38 (0·12)	0·98	+
Antistin	10·00	7·67 (0·13)	6·39 (0·19)	1·28	+
Histostab	9·97	7·40 (0·20)			
Chlorothen	8·70	9·50 (0·08)	8·35 (0·12)	1·15	⊕
Bromothen	8·63	9·64 (0·27)	8·26 (0·04)	1·38	⊕
Phenergan (promethazine)	9·08	8·93 (0·10)	7·96 (0·06)	0·97	+
Histantin		8·80 (0·18)			
Di-paralene (chlorcyclizine)	8·15	8·63 (0·08)	7·14 (0·25)	1·49	⊕
Trimeton (prophenpyridamine)	9·23	7·82 (0·24)	6·74 (0·05)	1·08	+
Chlor-trimeton (chlorprophenpyridamine)	9·16	8·82 (0·13)	7·89 (0·17)	0·93	+
Thenylene (methapyrilene)	8·85	8·63 (0·11)	7·64 (0·09)	0·99	+
Thephorin (phenindamine)	8·98	8·46 (0·21)	7·20 (0·18)	1·26	⊕
Phenothiazine Derivatives					
3015 RP	8·66	8·76 (0·17)	8·15 (0·16)	0·61	−
2987 RP	9·09	7·88 (0·05)	7·17 (0·08)	0·71	−
Halpern	9·02	7·95 (0·16)	7·17 (0·03)	0·78	+
3277 Phenergan	9·08	8·93 (0·10)	7·96 (0·06)	0·97	+
RP	9·06	8·86 (0·13)	8·26 (0·23)	0·60	−
Halpern	8·92	8·78 (0·23)	7·96 (0·10)	0·82	+
Lergigan	8·97	8·89 (0·16)	7·61 (0·16)	1·28	⊕
4460 RP	8·91	7·97 (0·07)	7·13 (0·27)	0·84	+
3356 RP	9·50	8·34 (0·11)	7·31 (0·04)	1·03	+
Halpern	9·64	8·27 (0·09)	7·31 (0·03)	0·96	+
3349 RP	9·61	6·50 (0·23)	5·79 (0·12)	0·71	+
4605 RP	9·02	7·60 (0·11)	7·06 (0·12)	0·54	−
4588 RP	8·96	7·81 (0·20)	7·16 (0·18)	0·65	+
3554 RP	11·23	8·18 (0·20)	7·81 (0·09)	0·37	−
3276 RP	9·52	8·25 (0·14)	7·39 (0·25)	0·86	+
3300 RP	9·13	7·00 (0·20)	6·26 (0·15)	0·74	+
Halpern	9·08	7·14 (0·07)	6·27 (0·07)	0·87	+
4560 RP	9·30	7·92 (0·11)	6·93 (0·20)	0·99	+
Benzhydryl (Chlorcyclizine) Series					
A-1198 ..	8·16	7·87 (0·07)	6·80 (0·06)	1·07	+
A-2285 ..	7·97	8·15 (0·09)	7·17 (0·05)	0·98	+
A-2342 ..	8·23	8·03 (0·09)	7·14 (0·04)	0·89	+
A-2688 ..	8·21	8·16 (0·11)	7·16 (0·23)	1·00	+
A-2824 ..	8·10	7·25 (0·22)	6·22 (0·10)	1·03	+
Chlorcyclizine	8·15	8·63 (0·08)	7·14 (0·25)	1·49	⊕
A-2917	Not titratable	7·09 (0·17)	5·93 (0·05)	1·16	⊕
A-4826	Not titratable	5·23 (0·15)	4·07 (0·14)	1·16	+
Phenindamine and Derivatives					
Phenindamine	8·98	8·46 (0·21)	7·20 (0·18)	1·26	⊕
Nu-1326 ..	7·71	6·39 (0·11)	5·26 (0·07)	1·13	⊕
Nu-1525 ..	8·66	5·71 (0·15)	4·61 (0·20)	1·10	+

increase in antagonist will be required to antagonize a ninefold increase in spasmogen concentration, since the increase in *contraction* is always much less than ninefold. This relationship between type of antagonism and (pA_2-pA_{10}) difference has been described by Schild (1947), and can be derived mathematically from the Mass Action equation of Gaddum (1937).

The frequency distribution of (pA_2-pA_{10}) differences for all the compounds tested (Fig. 2) shows two clearly defined peaks at 0.60 and 0.95. The latter peak most likely represents the competitive compounds, whereas the peak at 0.60 represents the non-competitive compounds. A method of dividing the compounds statistically into the two groups was devised by adding 0.95 to each pA_{10} value and then determining whether or not this sum differs significantly from the value for pA_2 by calculating the value of t (P=0.05). The criterion of competitive or non-competitive action denoted in the final column of Table II is based on this calculation.

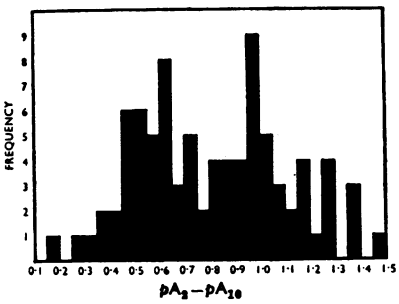


FIG. 2.—Frequency distribution of the values of pA_2-pA_{10}, showing peaks at 0.60 and 0.95.

Abnormally High (pA_2-pA_{10}) Differences

Fig. 2 shows that there is considerable spread of values for (pA_2-pA_{10}) above 0.95, the theoretical value for competitive antagonism. Some of these high values fall outside the calculated limits of deviation from 0.95, and such compounds are therefore theoretically non-competitive. On logical grounds, however, there is no justification for accepting such compounds as non-competitive, particularly as a large proportion of the proprietary antihistamines are in this category. As many of the antihistamines also antagonize ACh, a possible explanation of the abnormally high (pA_2-pA_{10}) values suggested itself, and the theory was to some extent substantiated by experimental evidence.

It was noted that mepyramine had a normal (pA_2-pA_{10}) difference, whereas that of diphenhydramine was high. It is well known that, although diphenhydramine shows powerful anti-ACh as well as antihistamine action, mepyramine is a relatively specific antihistamine. If, therefore, diphenhydramine molecules have an affinity for both histamine and ACh receptors, it might be that, at the relatively higher concentrations of diphenhydramine used in determining pA_{10} against

histamine, a higher proportion of diphenhydramine molecules are attracted to ACh receptors. There would therefore be increased competition between the two types of receptor for diphenhydramine molecules, so that the apparent amount of drug required to produce a given antagonism of histamine would be greater than if the drug were taken up solely by histamine receptors. Thus the pA_{10} value would be lower, and the (pA_2-pA_{10}) difference greater. Furthermore, if this theory is true, then it should be possible to restore the (pA_2-pA_{10}) difference to the theoretical 0.95 by determining pA_2 and pA_{10} values in atropinized Tyrode solution, since then the ACh receptors would already be blocked by the atropine, leaving all the diphenhydramine molecules free to combine with histamine receptors.

Accordingly, the pA_2 and pA_{10} values for mepyramine—normal (pA_2-pA_{10}) difference—and three antihistamines having high (pA_2-pA_{10}) differences were re-determined, using Tyrode solution containing 10^{-6} atropine sulphate, and the new (pA_2-pA_{10}) differences were compared with those previously obtained with normal Tyrode solution (Table III). With mepyramine the

TABLE III

EFFECT OF ATROPINIZED TYRODE ON THE (pA_2-pA_{10}) DIFFERENCE. (EACH pA VALUE QUOTED IS THE MEAN OF FOUR DETERMINATIONS)

Antihistamine	Normal Tyrode			Tyrode + Atropine SO_4 10^{-6}		
	pA_2	pA_{10}	(pA_2-pA_{10})	pA_2	pA_{10}	(pA_2-pA_{10})
Mepyramine ..	9·36	8·38	0·98	8·95	7·93	1·02
Diphenhydramine	8·14	6·78	1·36	7·68	6·52	1·16
Tripelennamine	9·00	7·64	1·36	8·49	7·33	1·16
Chlorcyclizine ..	8·63	7·14	1·49	7·98	6·90	1·08

value was unchanged, but with diphenhydramine, tripelennamine, and chlorcyclizine the original high values were reduced, though not to the theoretical 0.95. These results give some support to the theory of the origin of high (pA_2-pA_{10}) values. The slopes of the pA_2 and pA_{10} regression lines, determined in normal and atropinized Tyrode, differed significantly only for chlorcyclizine; that is, only with chlorcyclizine was the reduction of (pA_2-pA_{10}) difference significant. With chlorcyclizine and tripelennamine, but not diphenhydramine, the (pA_2-pA_{10}) difference, when determined in atropinized Tyrode, did not differ significantly from 0.95.

It is, therefore, considered justifiable to designate all compounds with abnormally high (pA_2-pA_{10}) difference as competitive antagonists of histamine. They are distinguished in Table II (last column) from the statistically proven competitive antagonists by the sign ⊕.

Duplicate Samples

Where more than one sample or proprietary preparation of the same compound was included, the pK_a values were always in good agreement, and there was no significant difference between the pA_2 values of eight compounds in which duplicate samples were tested. With regard to the type of histamine antagonism, discrepancies occurred between samples of RP 3277 and 2987. Whereas promethazine, lergigan and Halpern 3277 appeared to be competitive antagonists of histamine, the Rhone-Poulenc sample of 3277 was non-competitive. It was thought that this discrepancy might be explained by the latter sample containing a larger proportion of the (−)-isomer, since in compound 49/III/CN (Table II) the (−)-isomer is competitive and the (+)-isomer non-competitive. However, when examined polarimetrically, promethazine, RP 3277 and Halpern 3277 were all found to be optically inactive (Burke, private communication). Melting point determinations (Wragg, private communication) also failed to suggest any reason for the discrepancy. In comparison with a recent batch of promethazine (M. and B., batch 76, m.p. 233° C.), RP 3277 and Halpern 3277 were of reasonable purity (m.p. 232° C. and 231° C., respectively), while lergigan is slightly impure (m.p. 228° C.) and the original sample of promethazine very impure (m.p. 214–226° C.). It is strange, therefore, that the relatively pure RP 3277 is apparently non-competitive, while the less pure promethazine and lergigan are competitive.

Relation Between pA_2 and pK_a

Early studies on the proprietary antihistamines indicated a correlation between activity and dissociation constant. The most active antihistamine tested was bromothen which has a pK_a value of 8.63. Compounds with pK_a values below or above this were less potent, and above 8.63 the potency decreased inversely with the pK_a value. Unfortunately only one compound, chlorcyclizine, fell below pK_a 8.63, but this had a proportionately lower antihistamine potency than bromothen. From these data it was possible to construct, as a working hypothesis, a curve relating antihistamine potency to pK_a and resembling that obtained by Bell and Roblin (1942) for the sulphonamides (Fig. 3).

Determinations of antihistamine potencies more accurately in terms of pA units gave essentially the same picture for the proprietary antihistamines

as that obtained in the preliminary experiments. The investigation was then extended to a wide range of compounds all of which inhibited histamine-induced contractions of plain muscle. It was soon realized that these compounds must be screened not only for antihistamine potency but also to determine which were competitive antagonists of histamine, since it would be incorrect to correlate antihistamine potency with pK_a in compounds which were not competitive antihistamines. The results of this investigation have already been recorded in Table II, in which compounds with a (pA_2-pA_{10}) difference not significantly different from 0.95 are regarded as being competitive. Correlation coefficients between pA_2 and pK_a were then determined for the competitive and non-competitive members of each chemical group. These are summarized in Table IV. Assuming that a peak activity occurs at pK_a 8.6, coefficients have been calculated separately for compounds below 8.6 (positive correlation) and for compounds above 8.6 (negative correlation). As in the early experiments, the majority of compounds among the competitive antagonists had a pK_a value higher than 8.6. With the exception of Nu-1326, only the compounds related to chlorcyclizine had values below 8.6. The results show that there is a significant correlation between pA_2 and pK_a for the proprietary antihistamines with pK_a values above 8.6

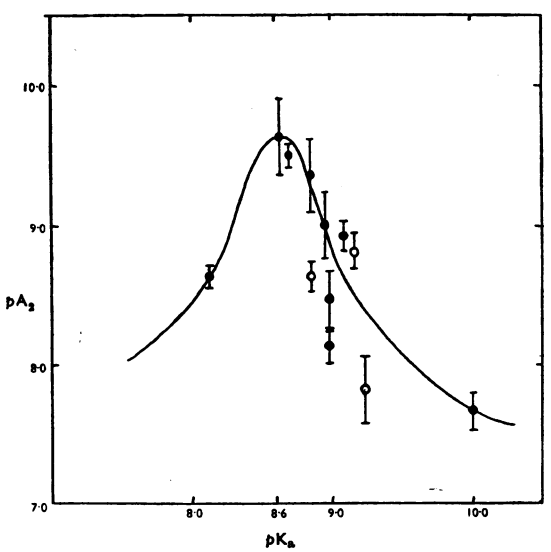


FIG. 3.—Curve of the relationship between pK_a and pA_2 for the proprietary antihistamines. (Vertical lines represent standard deviations of pA_2 values.) Solid dots—nine antihistamines upon which the curve was drawn. Open dots—three antihistamines (trimeton, chlor-trimeton, and thenylene) added after the hypothetical curve had been constructed.

TABLE IV
RELATIONSHIP BETWEEN pA_2 AND pK_a

Series	Correlation			
	No. of Compounds	Coefficient r	Tabulated r (P=0·05)	Significance
Competitive compounds				
Proprietary antihistamines (above pK_a 8·6)	11	−0·78	0·59	+
Benzhydryls (below 8·6)	6	+0·11	0·76	−
"Wilson" (above 8·6)	7	−0·069	0·72	−
Phenothiazines (above 8·6)	13	−0·51	0·55	−
"Methadone" (above 8·6)	10	−0·70	0·63	+
All competitive comps. (above 8·6)	41	−0·50	0·30	+
All competitive comps. (below 8·6)	7	+0·76	0·72	+
Non-competitive compounds				
"Wilson" (above 8·6)	11	+0·018	0·59	−
"Wilson" (below 8·6)	5	+0·30	0·84	−
Phenothiazines (above 8·6)	5	−0·16	0·84	−
"Methadone" (above 8·6)	9	+0·57	0·65	−
"Methadone" (below 8·6)	6	+1·15	0·76	+
All non-competitive comps. (above 8·6)	25	+0·11	0·40	−
All non-competitive comps. (below 8·6)	11	+0·43	0·59	−

(all of which were competitive), for the competitive compounds in the "methadone" series with pK_a values above 8.6, and for the total competitive compounds with pK_a values above and below 8.6. Among the non-competitive compounds, only one group, the "methadone" series below 8.6, showed significant correlation. The significance of correlation was never altered by inclusion or exclusion of the compounds with abnormally high (pA_2-pA_{10}) difference.

DISCUSSION

The evaluation of antihistamine potency in a wide range of compounds has emphasized the value of the pA method of assay, both as regards quantitative accuracy and the qualitative division of the compounds into groups representing competitive and non-competitive antagonists. When the frequency distribution of compounds according to the difference between pA_2 and pA_{10} values is plotted, two clearly marked peaks are obtained, one at pA 0.60, and the other at pA 0.95. The lower value represents fourfold, and the higher value ninefold, increase in the amount of antagonist required to inhibit a ninefold histamine increase. This latter reflects the molecule-for-molecule relationship which characterizes competitive antagonism, expressed mathematically in the Mass Action equation of Gaddum (1937). Any (pA_2-pA_{10}) value significantly lower than 0.95 must represent an antagonism which is non-competitive, and due to general spasmolytic action

on the plain muscle contraction rather than to a competition for histamine receptors. Some compounds have shown (pA_2–pA_{10}) differences significantly higher than 0.95, and it is suggested that these high values are related to those compounds which are competitive antagonists of ACh as well as of histamine, so that, at higher concentrations, some of the antagonist is "wasted" by being adsorbed on to ACh receptors. In support of this theory it has been shown that, in three of the compounds with high values, the pA_2–pA_{10} difference can be lowered by blocking ACh receptors with atropine. This multiple affinity for different types of receptor might explain the high values obtained by Guarino and Bovet (1949), who derived an equation which required a 45-fold increase of antagonist to balance a ninefold increase of active drug. This represents a (pA_2–pA_{10}) difference of 1.65. The highest value obtained for the antihistamines reported in this paper was 1.49 (that is, 30-fold) for chlorcyclizine.

The main purpose in dividing the compounds into competitive and non-competitive groups was to test the validity of a relationship between antihistamine potency and dissociation constant which had been previously observed in the proprietary antihistamines. The most potent antihistamine tested has a pK_a of 8.63, those above and below this value having lower activity, so that it was assumed as a working hypothesis that the relation between antihistamine potency and pK_a followed a curve, with a maximum activity at pK_a 8.6. This hypothesis has been tested on the results obtained from a wide range of spasmolytic compounds inhibiting histamine, by calculating correlation coefficients for pA_2 against pK_a for competitive and non-competitive compounds above and below pK_a 8.6. Taking all the compounds in each of these four groups, it has been shown that there is a significant negative correlation below pK_a 8.6 and a significant negative correlation above pK_a 8.6 for the competitive compounds. On the other hand, there is no correlation between pA_2 and pK_a in the non-competitive groups. When the compounds are divided into smaller, chemically related groups, the relationship does not always hold good, possibly because of insufficient numbers in some groups. These results strongly support the suggestion that the pA_2–pK_a relationship for antihistamine compounds follows a curve similar to that of Bell and Roblin (1942) for the sulphonamides.

The validity of this relationship has been tested by predicting the pA_2 values of three antihistamines, the dissociation constants of which were determined before their antihistamine potency. From the pA_2–pK_a curve plotted from data obtained with nine other proprietary antihistamines, the theoretical pA_2 value was read off according to the pK_a value for each compound. The predicted and experimentally determined values for pA_2 for the compounds were: prophenpyridamine, 8.12; 7.82 (0.24): chlorprophenpyridamine, 8.22; 8.82 (0.13): methapyrilene, 9.20; 8.63 (0.11). Figures in parentheses are standard deviations. This shows reasonably good agreement between predicted and actual values.

The division of a relatively large number of compounds into competitive and non-competitive antagonists of histamine has provided some interesting observations on the chemical structures required for histamine competition. Usually a two-carbon side-chain is important for specific antihistamine action, but duplicating this ethylamine side-chain as in compound 20 destroys the competitive action. Addition of other nitrogenous basic groups to the molecule (compounds 21, 22) also nullifies the action of the ethylamine chain, but in compounds 23 and 24, where the additional nitrogenous side-chain forms a closed ring, competitive antagonism is maintained.

Modification of the ethylamine side-chain is possible, however, within certain limits without destroying competitive action. The "methadone" series provides an illustration of the extent to which such modification can be made. Substituting a piperidine ring for the terminal amine does not affect the competition with histamine (compounds 50/II/CN, 50/I/CN, 50/I/COEt), but substituting a morpholine ring as in phenadoxone and related compounds destroys competitive action. The reduction of antihistamine and spasmolytic potency on substituting the morpholine group has previously been reported (Forbes and Marshall, 1951). Addition of a methyl group to one or other of the two carbon atoms of the side-chain has variable effects, apparently depending on the structure of the rest of the molecule. Thus, in compounds 49/IV/CN and 49/IV/COMe, substitution on the β-carbon atom renders the compound non-competitive, whereas in compounds 50/I/CN and 50/I/COEt histamine competition is maintained in the presence of the β-methyl substitution. It may be significant that compounds 50/I/CN and 50/I/COEt both have the piperidine nitrogen. In compounds 49/III/CN, 50/II/CN, 49/III/COOH and methadone itself, competitive action is maintained in the presence of α-methyl substitution, whereas compounds 49/III/COOEt, 49/III/COBu, and 50/II/COEt, also with α-methyl groups, are non-competitive. In the phenothiazine series, both the

α- and β-methyl derivatives (as in promethazine, 3277 and *iso*-promethazine, 4460) are competitive. Substitution by ethyl in the α-position (compound 4605) destroys histamine competition.

Obviously, all compounds with an ethylamine side-chain are not antihistamines, and the structure of the nucleus of the molecule plays an important part in establishing competitive antagonism. The "methadone" series provides an interesting illustration of this point, though it is impossible to establish any regular rule with regard to the function of the various substituents on the second (non-basic) side-chain in rendering the compound competitive or not. It appears that specificity for histamine is decided by a "balance" between the substituents on the ethylamine side-chain and on the rest of the molecule. For example, compounds 49/V/COEt, 49/III/COBu, 49/IV/COMe, and 50/II/COEt with a ketonic second side-chain are non-competitive, but the same ketonic side-chain associated with β-methyl and pyrimidine nitrogen substitution in the ethylamine chain (compound 50/I/COEt) or α-methyl substitution (methadone) renders the compound competitive.

The two stereo-isomers of 49/III/CN are interesting, the (+)-isomer being non-competitive and the (−)-isomer competitive. Unfortunately no other resolved compounds were available, so that it was impossible to establish whether (−)-rotation is always essential for histamine specificity in optically active compounds. An anomaly is seen with quaternized compounds. Compound 50/XII/COOEt ("methadone" series) was supplied as the methyl iodide, and was competitive; on the other hand, quaternization of promethazine (compound 3554) results in loss of competition for histamine.

Several apparent anomalies occur in the phenothiazine series. Of four samples of RP 3277 from three different sources, one is non-competitive and the other three competitive. The sample of lergigan, supplied originally under the formula of compound 4460, but since identified as identical with promethazine, was competitive. A similar difference in type of inhibition occurs between the two samples of compound 2987. These anomalies are not explicable on grounds such as difference in optical rotation or in purity.

Another unusual feature of the phenothiazine series is that compound 4560, with a three-carbon side-chain, is competitive. Here, the chlorine atom on the phenothiazine ring may be a factor "balancing" the extra carbon atom in the side-chain.

One group of compounds, the phenindamine series, deviates considerably from the typical antihistamine structure. Phenindamine itself, with a pK_a of 8.98, falls reasonably close to the pA_2–pK_a curve. It is a competitive antagonist, as also are the two related compounds Nu-1326 and Nu-1525. One would therefore expect Nu-1525 (pK_a 8.66) to be very potent, and Nu-1326 (pK_a 7.71) to have low antihistaminic potency, but the pA_2 of Nu-1525 is only 5.71, whereas that of Nu-1326 is 6.39. Although only three compounds in this series were available, one is tempted to suggest that the mechanism of antihistamine action of these compounds is different from that of other antihistamines, and is probably not a function of ionization.

The conclusions to be drawn from this study are that two factors are involved in determining antihistamine activity. First of all, qualitatively, the structure and shape of the molecule determines whether or not the compound is a competitive antagonist of histamine. The relative structure of both ring and side-chain appear to be important, and it is only possible, from observations on a relatively small number of compounds, to make tentative suggestions as to the limitations of structure capable of conferring antihistamine activity. The second factor is the basicity of the nitrogenous group of the molecule, which appears to exert a quantitative control on the potency of competitive inhibitors of histamine. Thus, if a compound possesses the right structure for antihistamine action, then the nearer its dissociation constant is to pK_a 8.6, the more potent it will be.

SUMMARY

1. A wide range of nitrogenous basic compounds has been screened for antihistamine potency by the pA method. Both pA_2 and pA_{10} were determined in order to distinguish competitive antagonists of histamine from non-competitive spasmolytics.

2. The dissociation constants (pK_a) of the compounds were measured by potentiometric titration, and a significant relationship between dissociation constant and antihistamine potency was established for those compounds which are competitive.

3. Factors concerning the relationship between chemical structure and antihistamine action are discussed in the light of the results obtained. It is suggested that a different mode of action operates with derivatives of phenindamine.

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