RELATIONSHIPS BETWEEN STRUCTURE AND ALBUMIN-BINDING OF AMINES TESTED WITH CROSSING-PAPER ELECTROPHORESIS

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INTRODUCTION

The interactions of physiological substances and drugs with blood proteins have been studied for decades with a great variety of methods. The results of these investigations, which have been reviewed by GOLDSTEIN[1], illustrate not only this variety, but also the many cases of discrepant results obtained by different authors or methods. This is due to many factors such as the complexity of the problem, the uncertainty of several methods, the instability of many protein-addition compounds. JERCHEL *et al.* introduced paper electrophoresis in this field[2] as well as its combination with the use of radioactive labelled test substances[3]. A relatively quick and easy method, crossing-paper electrophoresis, was published a few years ago by NAKAMURA and coworkers[4–9]. This method was used for the direct demonstration of the formation of loose addition compounds, *e.g.*, between enzymes and substrates. BICKEL AND MARINI-BETTÒLO[10] have reported recently on this technique and its use in the detection of interactions between drugs and blood proteins.

Owing to the simplicity of the method when it was once standardized, it was possible to test a great number of substances and thus to study possible relationships between the chemical structure of the substances and their formation of complexes with blood proteins.

METHOD

In our tests with crossing-paper electrophoresis, blood serum was run in the electric field so that it crossed a line of the test substance. A deformation of the albumin band at the crossing point means complex formation between the two substances, whereas if the band remains normal this indicates that no interaction occurs. The value of this method has been discussed in our recent paper[10].

In all experiments dog serum was used as protein. The results refer to the albumin fraction only.

EXPERIMENTAL

The technique and the conditions used have been described in detail previously[10].

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RESULTS

Some 75 substances were screened, and the results are listed in Table I. As all the substances contain nitrogen, they are grouped according to the degree of substitution of the amino group (primary, secondary, etc.), and the number of nitrogen atoms (*e.g.*, 1 N tert., 2 N sec.). The second column indicates whether interaction with blood albumin occurred (+) or not (—).

DISCUSSION AND CONCLUSIONS

The results obtained with crossing-paper electrophoresis indicate some basic relationships between albumin interaction (+) and the structure of the interacting substances.

All interacting substances contain one or more tertiary amino groups (see Table I, groups D, G, L, M, P, Q, R). No compound without a tertiary amino group shows interaction, *e.g.*, pure primary or secondary amines or quaternary ammonium salts (A, B, E, F, H, I); however, not all tertiary amines interact with albumin. Of these tertiary amines two groups can be distinguished: (1) all pure tertiary amines with three relatively short substituent groups (C); (2) some mixed amines, *i.e.*, tertiary amines with another amino group in the same molecule (K, L, N).

As these relationships seem to be independent of the presence or absence of other chemical groups, such as ester, ether, carbonyl, halogen groups, it can be concluded that the amino group is the decisive factor for interaction with albumin.

The affinity of the tertiary amino group to albumin is evident in our experiments. In contrast with this "proteinophilic" group, the non-tertiary amino groups are not inert, but clearly "proteinophobic". This fact is demonstrated not only by the lack of interaction with all non-tertiary amines (A, B, E, F, H, I), but also by the experiments with mixed amines (K, L, M, N). In many of these cases the introduction of a primary, secondary or quaternary nitrogen annuls interaction with albumin. As examples compare, *e.g.*, trasentine (D)–procaine (K), I.S. 2400 or 2422 (G)–I.S. 2324 (L), the first eight phenothiazines (Q)–I.S. 964 (L). In these mixed amines there is an antagonism between the "proteinophilic" tertiary and the "proteinophobic" non-tertiary groups.

However, the tertiary amino group becomes "proteinophilic" only if it is combined with another principle, as is demonstrated by the lack of interaction in the case of tertiary amines with small substituent groups (C).

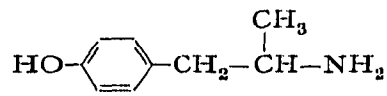
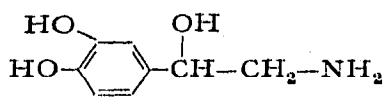
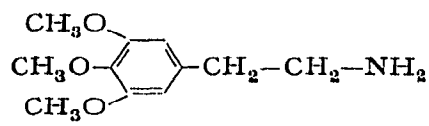
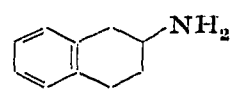
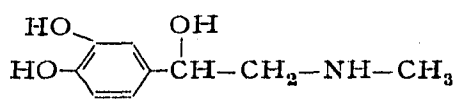
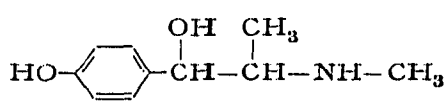
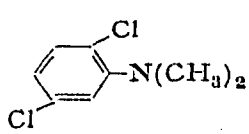
The relationship between structure and albumin interaction is less clear with compounds containing nitrogen in a cyclic system (O, P, R); however, in these cases interaction also occurs as a rule if a normal tertiary amino group is present in addition to the cyclic nitrogen (P, Q).

The fact that globulins are not considered in this study is of little importance as it is known that most interactions with blood proteins occur with the albumin fraction. The lack of interaction between the negatively charged blood albumin and the highly positively charged quaternary cations is a clear demonstration that these interactions are not due to mere electrostatic forces.

From the point of view of pharmacological classification, it can be said that, as a rule, the classical parasympatholytics, antihistamines, antiparkinsonian agents, phenothiazines and others, interact with blood albumin, whereas sympathomimetic amines, acetylcholine, urea, neuro-muscular blocking agents and others do not.

TABLE I

INTERACTION OF AMINES WITH BLOOD ALBUMIN

Substance tested		*Interaction*
(A) 1 N prim.		
Aniline	$C_6H_5-NH_2$	—
Benzylamine	$C_6H_5-CH_2-NH_2$	—
Phenylethylamine	$C_6H_5-CH_2-CH_2-NH_2$	—
Amphetamine	$C_6H_5-CH_2-\underset{\displaystyle CH_3}{\underset{\vert}{CH}}-NH_2$	—
Oxamphetamine		—
Noradrenaline		—
Mescaline		—
Tetrahydronaphthylamine		—
(B) 1 N sec.		
Di-*n*-amylamine	$C_5H_{11}-NH-C_5H_{11}$	—
Adrenaline		—
Suprifen		—
(C) 1 N tert. (short side chain)		
Triethylamine	$(C_2H_5)_3N$	—
1-Dimethylamino-2-chloropropane	$CH_3-\underset{\displaystyle Cl}{\underset{\vert}{CH}}-CH_2-N(CH_3)_2$	—
Diethylaniline	$C_6H_5N(C_2H_5)_2$	—
2,5-Dichloro-dimethylaniline		—

(continued on p. 469)

TABLE I (*continued*)

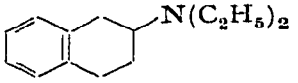
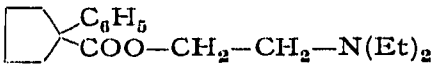
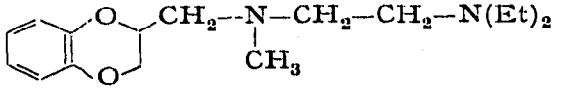
Substance tested		*Interaction*
Ethyl-benzyl-aniline	$C_6H_5-N<^{C_2H_5}_{CH_2C_6H_5}$	—
Methyl 4-dimethylamino-benzoate	$CH_3-OOC-C_6H_4-N(CH_3)_2$	—
Dibenamine	$Cl-CH_2-CH_2-N(CH_2C_6H_5)_2$	+ (?)
Benadryl	$(C_6H_5)_2CH-O-CH_2-CH_2-N(C_2H_5)_2$	+ (?)
Diethyl-tetrahydronaphthylamine		+ (?)
(*D*) *1 N tert. (long side chain)*		
Trasentine	$C_6H_5-CH-COO-CH_2-CH_2-N(Et)_2$	+
SKF 525-A	$C_3H_7-C(C_6H_5)_2-COO-CH_2-CH_2-N(Et)_2$	+
Parpanit		+
Myoparkil	$C_6H_5-CH_2-CH_2-C_6H_4-O-(CH_2)_3-N(Et)_2$	+
(*E*) *1 N quat.*		
Tetramethylammonium	$(CH_3)_4N^{\oplus}$	—
Tetraethylammonium	$(C_2H_5)_4N^{\oplus}$	—
Tetrapropylammonium	$(C_3H_7)_4N^{\oplus}$	—
Choline	$HO-CH_2-CH_2-\overset{\oplus}{N}(CH_3)_3$	—
Acetylcholine	$CH_3-COO-CH_2-CH_2-\overset{\oplus}{N}(CH_3)_3$	—
(*F*) *2 N prim.*		
Urea	$NH_2-CO-NH_2$	—
Cadaverine	$NH_2-(CH_2)_5-NH_2$	—
(*G*) *2 N tert.*		
I.S.2400		+

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TABLE I (*continued*)

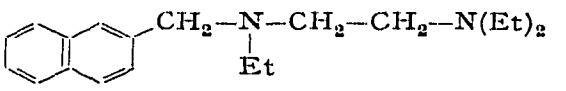
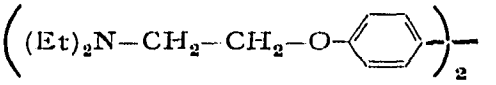
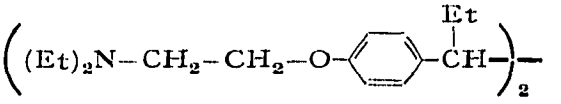
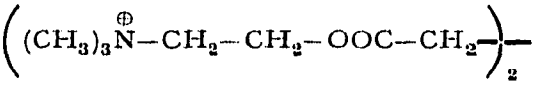
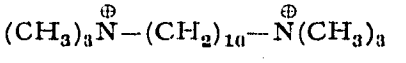
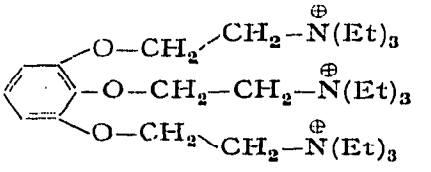
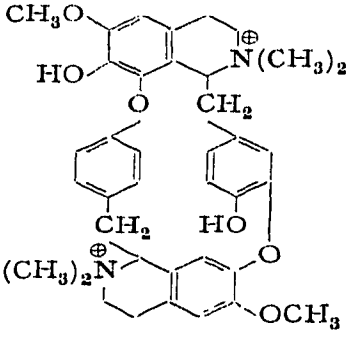
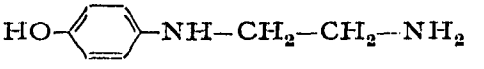
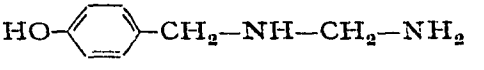
Substance tested		Interaction
I.S. 2422		+
Bis-(diethylamino-ethyl)-diphenyl 4,4-diether		+
Coralgil		+
(H) 2 or 3 N qual.		
Succinylcholine		—
Decamethonium		—
Gallammonium		—
d-Tubocurarine		—
(I) 1 N prim., 1 N sec.		
N-(β-Phenylethyl)-ethylenediamine		—
1-Amino-2-(*p*-hydroxy-anilino)-ethane		—
Amino-(*p*-hydroxyphenyl-methylamino)-methane		—

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TABLE I (*continued*)

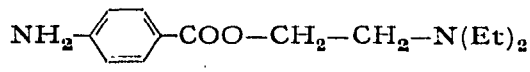
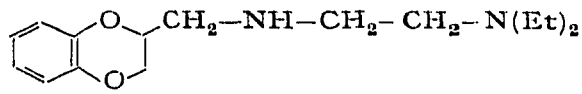
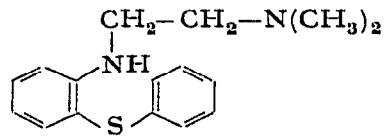
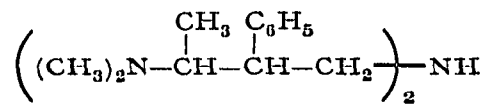
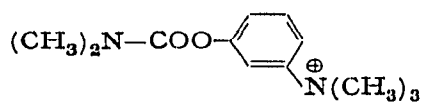
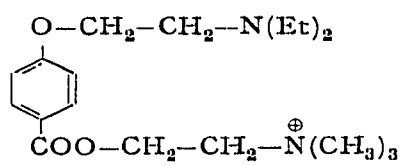
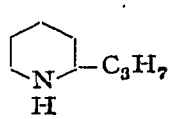
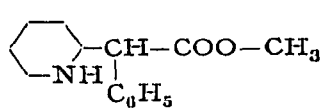
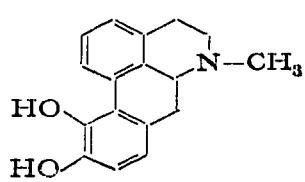
	Substance tested	*Interaction*
(*K*) *1 N tert., 1 N prim.*		
Procaine		—
(*L*) *1 N tert., 1 N sec.*		
Tetracaine		+
I.S.2324		—
I.S.964		—
(*M*) *2 N tert., 1 N sec.*		
Bis-(3-dimethylamino-3-methyl-2-phenyl-propyl)-amine		+
(*N*) *1 N tert., 1 N quat.*		
Prostigmine		—
I.S.444		—
(*O*) *1 N cyclic*		
Coniine		—
Ritalin		+ (?)
Apomorphine		—

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TABLE I (*continued*)

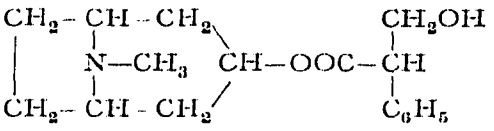
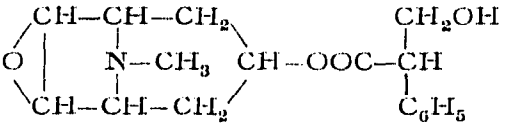
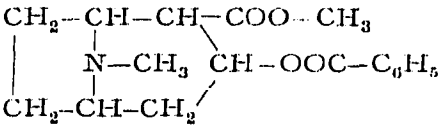
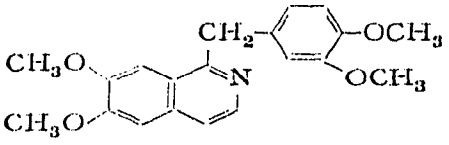
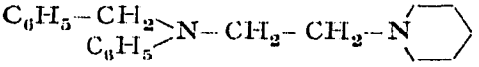
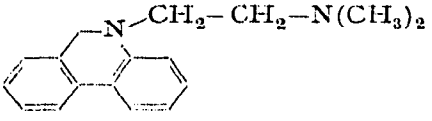
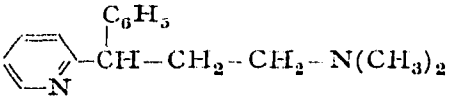
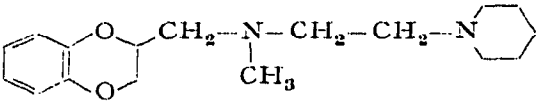
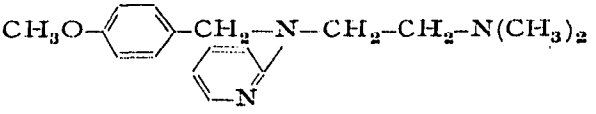
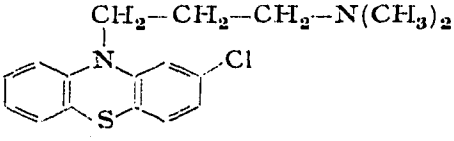
Substance tested		Interaction
Atropine		—
Scopolamine		—
Cocaine		+ (?)
Papaverine		+ (?)
(P) 1 N cyclic, 1 N tert.		
Allantan		+ (?)
N-(Dimethylamino-ethyl)-9,10-dihydro-phenanthridine		+
Trimethone		+ (?)
I.S.2294		+
Neoantergan		+
(Q) Phenothiazines (PHT-) 1 N tert.-cyclic, 1 N tert.		
Chlorpromazine		+

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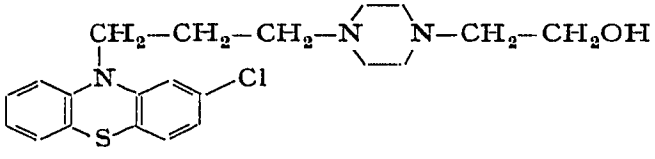
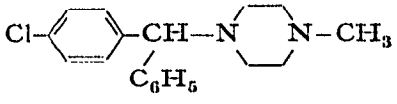
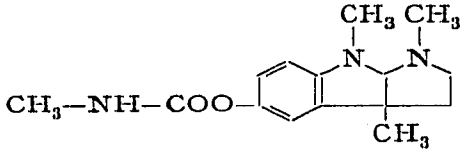
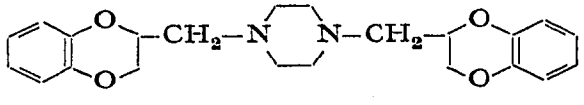
TABLE I (*continued*)

Substance tested		*Interaction*
Diethazine	PHT–CH_2–CH_2–$N(Et)_2$	+
Promethazine	PHT–CH_2–$CH(CH_3)$–$N(CH_3)_2$	+
Prophenamine	PHT–CH_2–$CH(CH_3)$–$N(Et)_2$	+
10-(Dimethylamino-acetyl)-PHT	PHT–CO–CH_2–$N(CH_3)_2$	+
10-(Diethylamino-acetyl)-PHT	PHT–CO–CH_2–$N(Et)_2$	+
10-(β-Diethylamino-propionyl)-PHT	PHT–CO–CH_2–CH_2–$N(Et)_2$	+
10-(β-Pyrrolidino-propionyl)-PHT	PHT–CO–CH_2–CH_2–N⟨pyrrolidine⟩	+
10-(γ-Dimethylamino-butyryl)-PHT	PHT–CO–CH_2–CH_2–CH_2–$N(CH_3)_2$	+
Trilafon		+
(R) 2 N cyclic		
Chlorocyclizine		+
Physostigmine		—
Dibozane		—

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SUMMARY

Some 75 nitrogen-containing substances have been screened with regard to their interaction with blood albumin by means of crossing-paper electrophoresis. The results show that there are definite relationships between the interaction and the chemical structure of the substances.

Only tertiary amines with at least one substantial radical interact, whereas primary and secondary amines and quaternary ammonium salts do not. With mixed amines interaction only occurs if the tertiary nitrogen "dominates" the other amino groups.

REFERENCES

1 A. GOLDSTEIN, *Pharmacol. Revs.*, 1 (1949) 102.
2 D. JERCHEL AND H. SCHEURER, *Z. Naturforsch.*, 8b (1953) 541.
3 D. JERCHEL, H. BECKER AND K. SCHMEISER, *Z. Naturforsch.*, 9b (1954) 169.
4 S. NAKAMURA, T. HOSODA AND T. UETA, *Proc. Japan Acad.*, 34 (1958) 742.
5 S. NAKAMURA AND T. UETA, *Naturwiss.*, 45 (1958) 569.
6 S. NAKAMURA, K. TAKEO, J. SASSAKI AND M. MURATA, *Nature*, 184 (1959) 638.
7 S. NAKAMURA, K. TAKEO, A. KATUNO AND S. TOMINAGA, *Clin. Chim. Acta.*, 4 (1959) 893.
8 S. NAKAMURA, K. TAKEO, K. TANAKA AND T. UETA, *Z. physiol. Chem.*, 318 (1960) 115.
9 S. NAKAMURA AND K. TAKEO, *Z. physiol. Chem.*, 320 (1960) 280.
10 M. H. BICKEL AND G. B. MARINI-BETTÒLO, *Sci. Repts. Ist. Super. Sanità*, 1 (1961) 105.