298. *Di-N-substituted 2-Halogenoethylamines. Part III.*[1,2] *N-2(or 3 or 4)-Chlorobenzyl-N-ethyl Derivatives: Synthesis, Reactivity, and Pharmacology.*

By J. F. Allen and N. B. Chapman.

The preparation of the group of compounds mentioned in the title, *o*-, *m*-, or *p*-Cl·C_6H_4·CH_2·NEt·CH_2·CH_2X,HX, with X = Cl, Br, or I, is described. The free amines in acetone–water (2 : 1) at 30° decompose by way of an ethyleneiminium ion, which is then mainly hydrolysed but may also react with the parent amine to some extent to yield piperazinium salts. By utilising chemically determined curves of the variation of ethyleneiminium ion concentration with time, and bioassays, anti-adrenaline and anti-noradrenaline activity are again (cf. Part II [2]) strongly correlated with ethyleneiminium ion concentration. The preparation of the picrylsulphonates of the derived ethyleneiminium ions is also described and measurements of their solvolysis in aqueous acetone at 30·0° are reported. The pharmacological properties of the halogeno-amines and of the ethyleneiminium ions are briefly reported. It is concluded that pharmacological differences between the 2-chloroethylamines studied probably depend as much on the efficiency of utilisation of ethyleneiminium ion *in vivo* as on the amount produced. The bromo- and iodo-compounds conform closely to the pattern of pharmacological properties established for the ethyleneiminium ion picrylsulphonates.

The object of the present paper is to extend the work previously reported by Chapman and James [1,2] to a series of compounds (I) in which the 1- or 2-naphthylmethyl radical, the persistent feature of the compounds studied by Chapman and James, is replaced by a

(I) *o*-, *m*-, or *p*-Cl·C_6H_4·CH_2·NEt·CH_2·CH_2X,HX

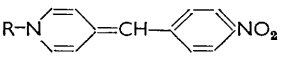
2-, 3-, or 4-chlorobenzyl group, with X = Cl, Br, I. Striking differences in pharmacological properties in this series, with X = Cl, consequent on the very slight change of structure resulting from a shift of position of nuclear chlorine, have been reported by Nickerson and Gump.[3] Thus 3-chloro-*N*-2-chloroethyl-*N*-ethylbenzylamine hydrochloride was more active than dibenamine [$N(CH_2Ph)_2$·CH_2·CH_2Cl,HCl] in the blockade of adrenaline, whereas the 4-isomer was less active. This has been confirmed by Graham,[4] who is again responsible for the pharmacological testing. In view of our previous findings on the dependence of anti-adrenaline activity on the power of ethyleneiminium ion formation, it

[1] The paper by Chapman and James, *J.*, 1953, 1865, is regarded as Part I.
[2] Part II, Chapman and James, *J.*, 1954, 2103.
[3] Nickerson and Gump, *J. Pharmacol.*, 1949, **97**, 25.
[4] Graham, *Brit. J. Pharmacol.*, in the press.

seemed to us of interest to study these compounds to see whether the structural changes were reflected in changes of reactivity, and hence, possibly, of anti-adrenaline activity.

The synthesis of the required materials, which does not appear to have been described, involved the interaction of a chlorobenzyl bromide with *N*-ethylaminoethanol to yield the corresponding di-*N*-substituted aminoethanol, which on treatment with phosphorus pentachloride, phosphorus tribromide, or phosphorus tri-iodide in chloroform gave the required halogeno-amines as salts (I) with halogen hydracid (cf. ref. 1). The picrylsulphonates of the derived ethyleneiminium ions were also isolated in an analytically pure state. A piperazinium salt, related to the piperazinium salts which would result from dimerisation of compounds of type (I), was prepared by a method given on p. 1484.

In an attempt to develop a satisfactory and specific colorimetric method of determining ethyleneiminium ions in dilute solution in water containing a little acetone, the interaction of 4-4′-nitrobenzylpyridine with halogenoethylamines was studied with a view to obtaining, by basification of the first formed quaternary salt, analytically pure compounds



(II)

of type (II), which show an intense blue colour (cf. Geissman, Hochman, and Fukuto [5]). Although the necessary quaternisation occurred readily in boiling ethanol it was too sluggish in aqueous acetone to provide a basis for colorimetric analysis.

Experimental

Preparation of Materials.—*2-(N-Chlorobenzyl-N-ethylamino)ethanols.* 4-Chlorobenzyl bromide [6] (51 g., 0·25 mole) was added during 1 hr. with stirring to 2-*N*-ethylaminoethanol (45 g., 0·50 mole) at 0°, and the reaction was completed by refluxing for 6 hr. The product was acidified with 6N-hydrochloric acid, unchanged 4-chlorobenzyl bromide was extracted with ether, and the aqueous layer was basified with 4N-sodium hydroxide. The colourless oil which separated was extracted with ether, dried (K_2CO_3), and after removal of ether gave 2-(N-4-*chlorobenzyl*-N-*ethylamino*)*ethanol* (89%), b. p. 88—92°/0·0006 mm., 142°/1 mm., n_D^{20} 1·5360 (Found: Cl, 16·8. $C_{11}H_{16}ClNO$ requires Cl, 16·6%) [*picrate* (from aqueous acetic acid), m. p. 118—119° (Found: C, 46·0; H, 4·2. $C_{17}H_{19}ClN_4O_8$ requires C, 46·1; H, 4·3; N, 12·7%); *picrylsulphonate* (from chloroform), m. p. 151·5—153° (Found: C, 39·9; H, 3·5; N, 10·8; S, 6·0. $C_{17}H_{19}ClN_4O_{10}S$ requires C, 40·3; H, 3·8; N, 11·1; S, 6·3%)].

Equimolar proportions of the bromide and aminoethanol in the presence of aqueous sodium hydrogen carbonate gave only a 56% yield of the required product.

2-(N-3-*Chlorobenzyl*-N-*ethylamino*)*ethanol*, b. p. 96°/0·005 mm., n_D^{20} 1·5333 (76%) (Found: Cl, 16·8%) [*picrate* (from aqueous acetic acid), m. p. 112·5—113·5° (Found: C, 45·6; H, 4·5; N, 12·3%); *picrylsulphonate* (from ethanol), m. p. 122—122·5° (Found: C, 40·6; H, 3·9; N, 11·0; S, 6·3%)], and the 2-*chloro-isomer* (85%), b. p. 86°/0·004 mm., n_D^{17} 1·5346 (Found: Cl, 16·3%) [*picrate*, m. p. 77—78° (Found: C, 45·8; H, 4·3%); *picrylsulphonate*, m. p. 126·5° (Found: C, 40·6; H, 3·8%)], were similarly prepared.

Chloro-compounds. A solution of the 4-chlorobenzylamine (20 g., 0·094 mole) in dry chloroform was added with stirring during 1 hr. to a suspension of phosphorus pentachloride (22 g., 0·10 mole) in chloroform at 0°. The mixture was kept at room temperature for 2 hr., chloroform was then removed at 25 mm., and the required 2-(N-4-*chlorobenzyl*-N-*ethylamino*)*ethyl chloride hydrochloride* (96%) was extracted from the residue with hot dry ethanol and precipitated by addition of the solution to dry ether; it had m. p. 145·5—146°, after recrystallisation from ethyl acetate (Found: C, 49·0; H, 5·9; N, 5·7; Cl, 39·6. $C_{11}H_{15}Cl_2N,HCl$ requires C, 49·2; H, 6·0; N, 5·2; Cl, 39·6%). The 3-*chlorobenzyl* (from dry ethanol–ether; 98%), m. p. 145—146° (Found: C, 49·3; H, 6·4; N, 5·1; Cl, 39·4%), and 2-*chlorobenzyl isomer* (from ethanol–ether; 98%), m. p. 144—144·5° (Found: C, 48·9; H, 5·8; N, 5·0; mobile Cl, 26·4%), were similarly prepared.

Bromo-compounds. Adding the 4-chlorobenzylamine (15 g., 0·07 mole) in dry chloroform to phosphorus tribromide (26 g., 0·1 mole) in chloroform at 0°, followed by boiling the mixture for

[5] Geissman, Hochman, and Fukuto, *J. Amer. Chem. Soc.*, 1951, **73**, 3313.
[6] Weizmann and Patai, *J. Amer. Chem. Soc.*, 1946, **68**, 150.

24 hr. and removal of chloroform, gave a product from which 2-(N-4-*chlorobenzyl*-N-*ethylamino*)-*ethyl bromide hydrobromide* (86%) was extracted with dry ethanol, and had m. p. 155—156° (from ethanol) (Found: C, 37·4; H, 4·5; N, 4·1; Br, 45·0. $C_{11}H_{15}BrClN,HBr$ requires C, 36·9; H, 4·5; N, 3·9; Br, 44·7%). The 3-*chlorobenzyl* (80%), m. p. 161·5—162·5° (Found: C, 37·0; H, 4·6; N, 3·8; Br, 45·0%), and 2-*chlorobenzyl isomer* (98%), m. p. 155° (Found: C, 37·3; H, 4·5; N, 4·0; Br, 44·7%), were similarly prepared.

Iodo-compounds. These were prepared as for the bromo-compounds, by using phosphorus tri-iodide in equimolar proportion with the alcohol. 2-(N-4-*Chlorobenzyl*-N-*ethylamino*)*ethyl iodide hydriodide* (75%), m. p. 128·5—130° (Found: C, 29·7; H, 3·3; N, 3·2; I, 56·7. $C_{11}H_{15}ClIN,HI$ requires C, 29·3; H, 3·6; N, 3·1; I, 56·3%), and its 3-*chlorobenzyl* (60%), m. p. 179·5—180° (Found: C, 29·7; H, 3·6; total halogen, 64·6. $C_{11}H_{15}ClIN,HI$ requires total halogen 64·1%), and 2-*chlorobenzyl isomer* (40%), m. p. 168—170° (decomp.) (Found: C, 29·2; H, 3·3; N, 3·0; I, 55·9%), were thus obtained.

Picrylsulphonates of ethyleneiminium ions. These were prepared by keeping solutions of the free bromoethylamines in acetone–water (2 : 1) at 30° for a time which gave the maximum ethyleneiminium ion concentration (previously determined by thiosulphate titration, see Fig. 5), extracting unchanged bromoethylamine with ether, and adding the aqueous layer to an excess of aqueous sodium picrylsulphonate at 0°. N-4-*Chlorobenzyl*-, m. p. 166—167° (Found: C, 41·5; H, 3·3; N, 11·5; Cl, 7·4; S, 6·7. $C_{17}H_{17}ClN_4O_9S$ requires C, 41·8; H, 3·5; N, 11·5; Cl, 7·3; S, 6·6%), N-3-*chlorobenzyl*-, m. p. 167—168° (Found: C, 41·7; H, 3·6; N, 11·3; S, 6·8%), and N-2-*chlorobenzyl*-N-*ethylethyleneiminium picrylsulphonate*, m. p. 154—155° (Found: C, 41·9; H, 3·1; N, 11·7; S, 6·7%), were crystallised from acetone–ether.

Recrystallisation of the 3-chloro-salt from chloroform gave crystals, of m. p. 134—135°, which contained chloroform (isocyanide test) (Found: C, 35·8; H, 3·2. $C_{17}H_{17}ClN_4O_9S,CHCl_3$ requires C, 35·6; H, 3·0%). The 4- and the 3-chloro-salts gave a mixture of m. p. 144—145°, thus confirming their individualities. The former had a molecular weight of 262 (cryoscopic in acetic acid) corresponding approximately to two particles per formula weight, thus confirming its salt-like character. All the picrylsulphonates consumed 0·96 mole of thiosulphate per mole at ~—80°, and about 0·93 mole at room temperature.

Piperazinium salts. 1,4-Diethyl-1,4-dimethylpiperazinium di-iodide was obtained by interaction of 1,4-diethylpiperazine and methyl iodide and had m. p. 240° after crystallisation from methanol. Smith *et al.*[7] give m. p. 240°. 1,4-*Di*-2-*chlorobenzylpiperazine*, obtained by interaction of 2-chlorobenzyl chloride and piperazine hydrate in ethanol at the b. p. for 2 hr., followed by basification, had m. p. 101—102° (from ethanol) (Found: C, 64·4; H, 5·9. $C_{18}H_{20}Cl_2N_2$ requires C, 64·5; H, 6·0%) [*dihydrochloride*, m. p. 260—262° (Found: ionic Cl, 17·0. $C_{18}H_{20}Cl_2N_2,2HCl$ requires ionic Cl, 17·4%)].

Miscellaneous compounds. Heating an ethanolic solution of *N*-ethyl-*N*-2′-iodoethyl-1-naphthylmethylamine hydriodide and 4-4′-nitrobenzylpyridine at the b. p. gave first 4-4′-nitrobenzylpyridinium iodide which was filtered off, and on addition of ether to the filtrate 1-2′-(N-*ethyl*-N-1″-*naphthylmethylamino*)*ethyl*-4-4′-*nitrobenzylpyridinium iodide*, m. p. 199—200° (Found: C, 59·1; H, 4·9; N, 7·3; I, 22·9. $C_{27}H_{28}IN_3O_2$ requires C, 58·6; H, 5·1; N, 7·6; I, 22·9%). Treating this compound with alkali gave a blue substance of type (II), but this could not be obtained analytically pure. However, a similar procedure using 2-chloro-*N*-ethyl-*N*-2′-iodoethylbenzylamine hydriodide gave, without isolation of the quaternary salt, a *compound*, $C_{23}H_{24}ClN_3O_2$, of type (II) [from chloroform–light petroleum (b. p. 40—60°)], m. p. 65° (Found: C, 67·2; H, 5·6; N, 10·1. $C_{23}H_{24}ClN_3O_2$ requires C, 67·4; H, 5·9; N, 10·3%).

Procedure.—This was as described in Part II,[2] save that determinations of acid were done by direct titration with sodium hydroxide. The efficiency of the ether-extraction used to remove unchanged halogeno-amine was tested by shaking an aqueous acetone (1 : 2) solution of the halogeno-amine with ether in the usual way, immediately after the amine had been liberated from its salt. Halide ion in the aqueous layer was then determined by the Volhard method and, within experimental error, corresponded to the combined hydrohalide only of the original salt. It was also shown that 1,4-diethyl-1,4-dimethylpiperazinium di-iodide did not consume thiosulphate. It is therefore very unlikely that thiosulphate would be consumed by any of the possible dimerisation products of the halogeno-amines being studied. Moreover, blank experiments showed that acid formed by hydrolysis of the ethyleneiminium ions produced did not affect the thiosulphate titres.

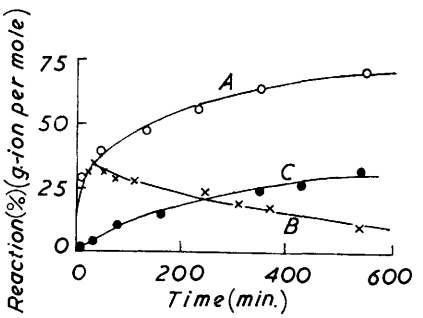
[7] Smith, Curry, and Eifert, *J. Amer. Chem. Soc.*, 1950, **72**, 2969.

Solvolysis of ethyleneiminium picrylsulphonates. Aliquot parts of a solution of the picrylsulphonate in acetone–water (2 : 1), maintained at 30·0° in a thermostat bath, were withdrawn at intervals and run into an excess of standard aqueous sodium thiosulphate. After 10 min., the residual thiosulphate was titrated with standard iodine. Appropriate blank determinations were made to allow for any interference by acetone with the titration. First-order plots gave satisfactory straight lines. The rate coefficients obtained from the slopes of these lines are included in Table 2.

Discussion

Figs. 1, 3, and 4 show the behaviour of the 4-, 3-, and 2-chloro-*N*-2-chloroethyl-*N*-ethylbenzylamine respectively. The curves are of the same general form as those previously

Fig. 1. *Decomposition of* 2-(N-4-*chlorobenzyl*-N-*ethylamino*)*ethyl chloride at* 30° *in aqueous acetone.*



A, Chloride ion liberated. B, Ethyleneiminium ion formed. C, Hydrogen ion formed.

Fig. 2. 2-(N-3-*Chlorobenzyl*-N-*ethylamino*)*ethyl chloride. The curve shows concentration of ethyleneiminium ion as a percentage of the maximum. Points represent bio-assays; open circles: against noradrenaline, full circles: against adrenaline. Both refer to aqueous acetone solutions at* 30°.

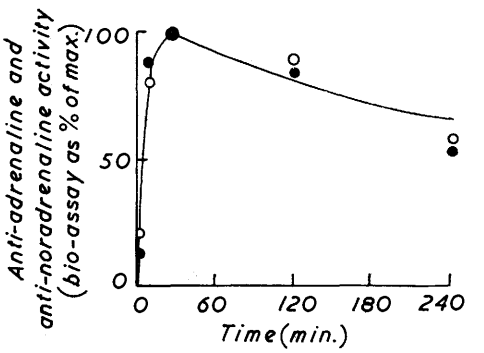
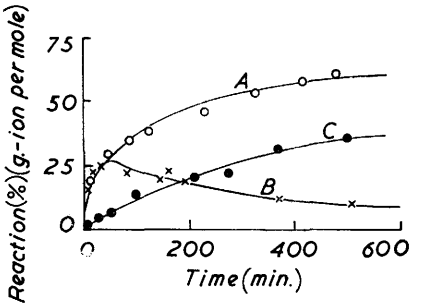
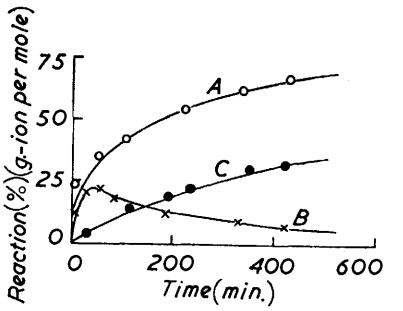


Fig. 3. *Decomposition of* 2-(N-3-*chlorobenzyl*-N-*ethylamino*)*ethyl chloride at* 30° *in aqueous acetone.*

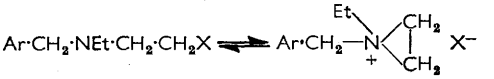


For Figs. 3—5: *A, Halide ion liberated. B, Ethyleneiminium ion formed. C, Hydrogen ion formed.*

Fig. 4. *Decomposition of* 2-(N-*chlorobenzyl*-N-*ethylamino*)*ethyl chloride at* 30° *in aqueous acetone.*



established for the 2-chloroethylamines studied by Chapman and James.[2] We interpret them, together with Fig. 5, as indicating that the essential step in decomposition is formation of the ethyleneiminium ion thus:



and that this is followed by decomposition of the ion, mainly but not exclusively by hydrolysis. Since the solvolysis of pure ethyleneiminium ions in 2 : 1 acetone–water at

30° shows regular first-order kinetics, it is unlikely that the hydrolysis products cause important side-reactions in these cases, and dimerisation of the halogenoethylamine seems therefore to be the most likely side-reaction (cf. Chapman and James [1]). It is clear that the time necessary for the attainment of maximal ethyleneiminium-ion concentration differs little for the three compounds, but the maximum value is in the order *para* > *meta* > *ortho* whereas the anti-adrenaline and anti-noradrenaline activities of the salts are in the order *meta* > *ortho* > *para*. In Fig. 2 the close agreement between chemical and biological assays of preformed ethyleniminium ion is exemplified for the *para*-compound

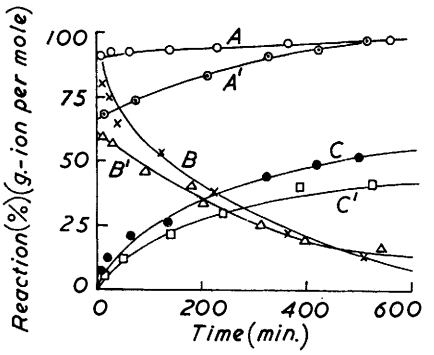


FIG. 5. *Decomposition of* 2-(N-4-*chlorobenzyl*-N-*ethylamino*)*ethyl bromide and iodide at* 30° *in aqueous acetone.*

A, B, C, refer to the bromide, and A', B', and C' to the iodide.

and similar observations have been made by Graham [4] for the other two compounds. We conclude that the relatively small structural change involved in shifting the position of the chlorine atom causes significant differences in reactivity as measured by the maximal ethyleneiminium-ion concentration (Table 1) but that the pharmacological differences

TABLE 1. *Reactions and properties of* Ar·CH_2·NEt·CH_2·CH_2X,HX.

X	Max. propn.* of X liberated (%) (after 500 min.)	Max. propn.* of ethyleneiminium ion formed (%)	Anti-adrenaline activity † (micromole/kg.)	Anti-noradrenaline activity † (micromole/kg.)
		4-Chlorobenzyl		
Cl	71	36	36·6	39·6
Br	98	81	3·5	5·3
I	95	60	4·1	4·9
		3-Chlorobenzyl		
Cl	61	26	4·9	5·0
Br	95	75	2·1	2·2
I	90	52	1·6	1·4
		2-Chlorobenzyl		
Cl	70	23	23·9	22·1
Br	98	75	2·1	2·6
I	93	52	1·5	2·1

* G.-ion/mole. † $E.D._{50}$ in spinal rats.

TABLE 2. *Properties of* N-*benzyl*-N-*ethylethyleneiminium picrylsulphonates.*

	Anti-adrenaline activity * (micromole/kg.)	Anti-noradrenaline activity * (micromole/kg.)	10^3k †
4-Chlorobenzyl	1·22	1·71	4·3
3-Chlorobenzyl	0·85	1·16	3·9
2-Chlorobenzyl	0·66	0·98	7·7

* $E.D._{50}$ in spinal rats. † k is the rate coefficient for first-order solvolysis in aqueous acetone at 30° in $min.^{-1}$.

observed when the compounds are tested as salts are not correlated with the extent of ethyleneiminium-ion formation from the free bases in aqueous acetone. Moreover, the pharmacological activities of the three ethyleneiminium ions when administered as picrylsulphonates run in the order *ortho* > *meta* > *para* (Table 2). The salt-like character of these picrylsulphonates has been confirmed by molecular-weight determination in one

case (cf. p. 1484) and this together with their m. p.s and chemical properties make it safe to assume that their aqueous-acetone solutions contain free ethyleneiminium ions and picrylsulphonate ions, the latter having no significant pharmacological action in the amounts used (Graham [4]). We may therefore ascribe the differences in pharmacological activity observed with the 2-chloroethylamines, when administered as their hydrochlorides, in part at least to differing efficiencies of conversion into the relevant ethyleneiminium ion *in vivo*. This view is confirmed by measurements of the rates of solvolysis in 2 : 1 acetone–water at 30° of the ethyleneiminium ions under discussion, for which an order *ortho* > *meta* ~ *para* is observed (Table 2). If these relative rates may be taken as a measure of the relative intrinsic alkylating powers of the ions *in vivo*, then the observed anti-adrenaline activities of the ethyleneiminium ion picrylsulphonates of the *ortho*- and *para*-compounds would be expected to show roughly the observed pattern if it is assumed that the essential biochemical feature of the anti-adrenaline activity is alkylation of appropriate groups. Harvey and Nickerson [8] have provided experimental evidence that the groups undergoing alkylation are thiol groups, but a more recent examination of the problem by Belleau [9] leads to the view that it is more likely that carboxylate or phosphate anionic groups are involved.

As a consequence of his interesting theory of the anti-adrenaline activity of 2-halogenoethylamines, Belleau [8] concluded that the *para*-chloro-compound should be inactive, whereas the *ortho*- and the *meta*-compound should be active. The results assembled in Tables 1 and 2 support this view to the extent that on any showing the *p*-compound is always the least active of the three, but the differences are quantitative rather than qualitative; and, if the activities of the ethyleneiminium picrylsulphonates are considered, then the maximum activity ratio is ~2 : 1. In our view the pharmacological properties of the pure ethyleneiminium ions are more suitable for correlation with structure than those of the *N*-substituted 2-chloroethylamine salts for reasons already given.

The behaviour of the bromo- and iodo-compounds containing the 4-chlorobenzyl residue, which are typical of this group of compound, is displayed in Fig. 5. The pattern is broadly similar to that previously observed apart from some depression of the reactivity of the iodo-compounds. The differences in pharmacological activity of bromo- and iodo-compounds are very slight, and, except for the *meta*-compounds, the chloro-compounds are, as is often the case, some ten times less active than the bromo- and iodo-compounds (cf. Table 1). The activity ratios for bromo- and iodo-compounds, when the less active *p*-compounds are compared with the almost equi-active *o*- and *m*-compounds, are very similar to those for the ethyleneiminium ion picrylsulphonates. This is understandable in terms of the rapid and almost complete conversion of the bromo-compounds into ethyleneiminium ion *in vitro* (see Fig. 5 for an example), and to a smaller degree for the iodo-compounds.

In this work further evidence is provided, from the results of direct administration and assay of solutions containing known amounts of ethyleneiminium ion, that this ion is the pharmacologically active species formed from each of a group of 2-halogenoethylamines showing anti-adrenaline and anti-noradrenaline activity. Moreover, apparent differences of pharmacological activity caused by small structural changes are shown to be due partly to the intrinsic properties of the relevant ions, but also, it seems probable, to differences in the efficiency of conversion of the 2-chloroethylamine salts into ethyleneiminium ions and of utilisation of the ions *in vivo*.

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[9] Belleau, *Canad. J. Biochem. Physiol.*, 1958, **36**, 731.