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THE ETHYLENEIMINIUM ION AS THE ACTIVE SPECIES IN 2-HALOALKYLAMINE COMPOUNDS

BY

J. D. P. GRAHAM

From the Department of Pharmacology, Welsh National School of Medicine, Cardiff

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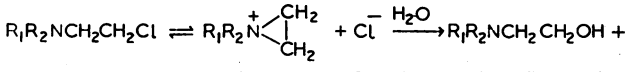
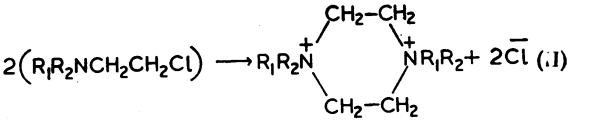
Certain 2-haloethylamine compounds which exert antagonism to adrenaline and noradrenaline of the type shown by dibenamine have been examined. The compounds were *N*-ethyl-*N*-chlorobenzyl-2-chloroethylamine hydrochlorides in which the Cl is in the *p*-, *o*-, and *m*- positions of the benzene ring, and the three related ethyleneiminium picrylsulphonates. In addition the Cl- and Br- compounds of the *N*-ethyl-*N*-9-fluorenyl-2-haloethylamine hydrohalide series were examined. For the haloethylamines, a curve was provided which showed the relation between probable production and decay of ethyleneiminium ion in neutralized solution with time. A correlation was established between the shape of this curve and the curve relating anti-adrenaline and anti-noradrenaline activity of samples of solutions of these compounds treated in an identical way. The relation to antagonism of histamine and 5-hydroxytryptamine was not so exact and varied with species.

The ethyleneiminium ions of the three chlorobenzyl compounds exerted a typical long-lasting non-competitive antagonism to the pressor actions of adrenaline and noradrenaline. They also antagonized the effect of 5-hydroxytryptamine and histamine on blood pressure. The ED50 of the ion when administered as ethyleneiminium picrylsulphonate was always less than the ED50 of the same ion when administered in the form of 2-haloethylamine. There was, therefore, a variable loss of effective ion during transformation *in vivo*. The observations throw some light on the importance of the substituent groups on the nitrogen atom for effectiveness. It is concluded from these results and a discussion of other work that 2-haloethylamine compounds form an ethyleneiminium ion in neutral solution, which is the pharmacologically active species in effective compounds. It is a necessary but not a sufficient condition that a molecule should be an alkylating agent for this type of antagonism to be present, but the importance of the substituent groups on the N atom is equally great because not all ethyleneiminium ions are effective antagonists.

2-Chloroethylamine derivatives such as dibenamine have been shown to undergo the following reaction (I) when in solution in aqueous media at physiological *p*H, an unstable cyclized chemical species, the *N*-disubstituted ethyleneiminium cation being formed:



Nickerson, Nomaguchi, and Goodman (1946) attributed the specific pharmacological activity of this type of compound to the formation of such an ion, by analogy with the known reactions of the structurally related nitrogen mustards. Nickerson and Gump (1949) have shown that the hydrolysis products in such reactions as (I) are pharmacologically inactive. Intermediate products, such as the dimer which might be formed (II), were prepared and also found to be inactive.



It is theoretically possible for hydrogen chloride to be eliminated from the chloroethyl group with formation of a vinylamine (III), but it is improbable that such intermediates are formed from active 2-haloethylamines when in buffered solution, and there is some evidence to the contrary.

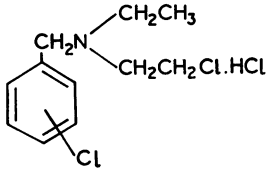
Such a compound would probably not react with sodium thiosulphate. Chapman, James, Graham, and Lewis (1952) reported the isolation and chemical characterization of the ethyleneiminium

ion derived from *N*-methyl-*N*-2-naphthylmethyl-2-bromoethylamine as a picrylsulphonate, but as this compound was insoluble in aqueous media it was unsuited to biological investigation. Accordingly lines of investigation were followed which did not involve the isolation of active cations from the parent compounds.

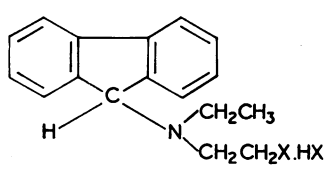
It was known that primary alkyl fluorides undergo substitution with much less ease than do other alkyl halides. A series of *N* - alkyl - *N* - 1 - naphthylmethyl - 2 - halo - ethylamines was prepared in which the halogen was Cl, Br, I or F (Chapman, James, Graham, and Lewis, 1951; Chapman and James, 1953) and it was shown that the fluorine compounds were pharmacologically and chemically inert, whereas the other halogen compounds were active. Mobility of the halogen is thus necessary for adrenergic blocking activity (Graham and Lewis, 1952, 1953). Chapman *et al.* (1952) and Chapman and James (1954) studied the kinetics of decomposition of some of these compounds in initially neutral solution and published curves relating the amount of thiosulphate consumed with the passage of time. Sodium thiosulphate reacts rapidly and stoichometrically with the ethyleneiminium ion; its consumption has been generally accepted as a measure of the amount of such ion in a mixture of products. The time relations of the release of halogen in the reaction mixture, the amounts of thiosulphate consumed, and the release of hydrogen ion were determined and plotted in graphic form. The pharmacologically inert fluorine-containing compounds were chemically inert and no halogen was released or thiosulphate consumed. In this respect, therefore, the conditions which determine cation formation also determine pharmacological activity. The presence and amount of anti-adrenaline and anti-histamine activity was determined in solutions of *N*-ethyl-*N*-1 - naphthylmethyl - 2 - haloethylamine (halogen=Cl, Br or I), prepared and handled in a manner identical to that used for the solutions in which chemical reactivity had been studied. It was thus possible to relate the formation and loss of a thiosulphate-consuming species (ethyleneiminium ion) in these solutions with specific pharmacological activity. It was clear that antagonism to adrenaline and histamine and consumption of thiosulphate ran a parallel course in these solutions as they were allowed to age (Graham and Lewis, 1954).

To exclude the possibility of this relationship being peculiar to these particular haloethylamine compounds it was decided to extend the examination to include further compounds, the *N*-ethyl-*N*-chlorobenzyl-2-chloroethylamines (IV) with Cl in the *ortho-* (JFA14), *meta-* (JFA11), or *para-* (JFA8) position of the benzyl residue, and *N*-ethyl-*N*-9-fluorenyl-2-haloethylamines (V), in which the halogen was Cl (AT_1) or Br (AT_2).

(IV)

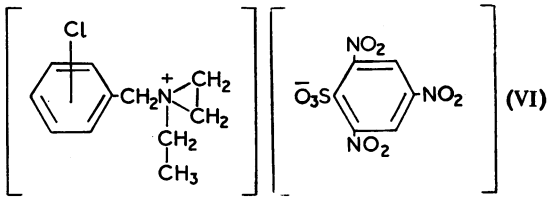


(V)



Nickerson and Gump (1949) refer briefly to two of these compounds, JFA11 and JFA8, and note that the former is more active. Kerwin, Herdegen, Heisler, and Ullyott (1950) describe the synthesis of compound AT1, and Kerwin, Ullyott, Fellows, and Macko (1949) state that it inhibits the pressor response to adrenaline in cats and is more active than dibenamine.

As this line of enquiry came to an end the ethyleneiminium ions derived from the three *N*-ethyl-*N*-chlorobenzyl-2-chloroethylamines became available in stable soluble form as picrylsulphonates (VI). They were given the code numbers P_1 (*p*-chloro-), P_2 (*m*-chloro-), and P_3 (*o*-chloro-) cations.



(VI)

Details of the synthesis of these compounds and of the kinetics in solution await publication (Allen and Chapman; see Allen, 1956).

This report, therefore, includes the results of extending the enquiry, using the technique described by Graham and Lewis (1954), and the examination of the specific activity of the ethyleneiminium ions derived from selected active halogenoethylamine compounds. A preliminary communication has been made by Graham (1957).

Methods

Chemical.—The chemical investigation was carried out by Allen and Chapman. Briefly, it consisted of the preparation of 200 ml. of 0.02 M solution of the compound in acetone-water (2:1). The finely powdered salt (4 mM.) was dissolved in a mixture of water (50 ml.) and acetone (132 ml.) at 30°, 10 ml. of 0.4 M solution of NaOH (4 mM. NaOH) added.

the solution made up to 200 ml. with water, shaken and kept at 30°. Aliquots were withdrawn at intervals and washed twice with ether to remove unchanged parent compound. Free halide, H ion, and ethyleneiminium ion were then determined. The % of the theoretically possible reaction (as g.-ion/mole reactant, maximum possible ion 0.004 mole) was plotted against time.

In addition the rate of loss of ion was determined when the aqueous phase after ethereal extraction of the mixture was kept on ice for periods of 10 to 72 min. This observation was made on mixtures of various ages up to 4 hr. and the loss was found to be negligible over a period of 60 min.

Pharmacological.—The solutions used were prepared in a manner identical to the above but adapted to give 10 ml. volume. 0.2 mM. of the compound was dissolved in 3 ml. water plus 6 ml. acetone, and 1 ml. N/5 solution of NaOH added, made up to 10 ml. volume and kept at 30°. At the selected time the mixture was extracted twice with 2 vol. of ether and the watery phase stored on ice while the ED50 was determined. Ten dogs were used for the solutions of that age where peak activity was found by preliminary trial (the reference point plotted as 100% activity) and 5 for the solutions of other ages examined. Five compounds were examined in this way. The dogs were atropinized, anaesthetized with 30 mg./kg. pentobarbitone sodium intravenously, and the blood pressure recorded. Repeated injections of (−)-adrenaline 4 μg./kg., equimolar amounts of (−)-noradrenaline and of histamine, and in some dogs 5 times this amount of 5-hydroxytryptamine (5-HT) were made into the jugular vein. At suitable intervals a dose of the treated solution of haloethylamine was injected. This was allowed to act for 15 min. and the standard injections repeated in random order. It had previously been shown that the parent compounds in solution exerted the maximum effect of any given dose in 15 min. or less and maintained this effect for at least 4 to 6 hr. The action of the individual doses was assumed to be cumulative. The amount given in mg./kg. weight of dog was recorded in terms of amount of parent compound present initially in the solution (0.02 M). The experiments were allowed to proceed until non-competitive reversal of the pressor responses was obtained, and, when possible, until partial recovery was noted. Less than 1% loss of activity occurs in an ice-cold solution of "ion" kept for 1 hr. Fresh solutions were prepared for subsequent stages of the experiment. The pharmacological activity of solutions of different ages was expressed as % of the mean activity measured at the age when peak activity was found to be present.

The ethyleneiminium picrylsulphonates were dissolved in a minimal volume of acetone (0.1 ml. for rats) and diluted as needed with saline. The ED50 for each of the 5 compounds and the 3 ethyleneiminium picrylsulphonates was determined against the 4 agonists in groups of 5 dogs and in 30 male atropinized spinal hooded rats pretreated with 5 mg./kg. of hexamethonium intravenously. Antagonism to adrenaline (1 μg./kg.) and to noradrenaline (0.5 μg./kg.) was measured together; antagonism to 5-HT (10 μg./kg.) was determined on different rats. Histamine was excluded from this series. Five rats were used for each determination and the parent compounds were similarly investigated for comparison.

The effect of Na picrylsulphonate by itself and on the responses to the four agonists was determined over a wide range of doses, in 3 dogs and 10 spinal rats, as a necessary preliminary to this part of the work.

RESULTS

The Relationship Between Ion Production and Pharmacological Activity

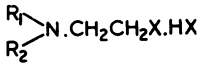
Some properties of the compounds examined are shown in Table I, which was compiled from information supplied by Allen and Chapman.

Compounds JFA8, JFA11, and JFA14 were freely soluble in water. Compounds AT1 and

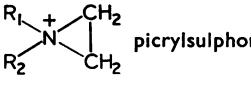
TABLE I

THE STRUCTURE, CODE NUMBERS AND MOLECULAR WEIGHTS OF THE COMPOUNDS AND IONS REFERRED TO IN THE TEXT

Solubility of AT_1 and AT_2 in water is poor, but they are soluble in acetone and this may be diluted 10 times with water before a precipitate appears. The ions P_1 to P_3 are similarly insoluble in water but soluble in acetone and may be diluted 19 times with water before precipitating. The last column shows the time of peak concentration of ion found by titration with thiosulphate in initially neutralized solutions of the haloethylamines and the maximum conversion to ion as a % of the theoretically possible figure.



R_1	R_2	X	Code No.	M.W.	Vol. of Water Dissolving 1 g. (ml.)	Max. Conversion to Ion as % of Theoretical Maximum
Et	*p*-Chlorobenzyl	Cl	JFA8	268·6	3·42	36·0 at 30 min.
,,	*m*-Chlorobenzyl	Cl	JFA11	268·6	6·34	26·1 at 30 min.
,,	*o*-Chlorobenzyl	Cl	JFA14	268·6	1·50	22·0 at 45 min.
,,	9-Fluorenyl	Cl	AT1	308·2	Sol. very poor	7·0 at 15 min.
,,	9-Fluorenyl	Br	AT2	397·2		40·0 at 0·5 min.



R_1	R_2		Code No.	M.W.	Vol. Acetone Dissolving 1 g. (ml.)	Max. Conversion to Ion as % of Theoretical Maximum at Zero Time
Et	*p*-Chlorobenzyl		P1	488·9	77	100
,,	*m*-Chlorobenzyl		P2	488·9	50	100
,,	*o*-Chlorobenzyl		P3	488·9	45	100

AT2 were relatively insoluble, but solutions in small volumes of acetone were easily diluted with water. The picrylsulphonates (P_1 to P_3) were also rather insoluble in water but readily soluble in acetone and did not easily come out of solution on dilution with water or saline. The amount of ion formation actually found on titration was recorded as a % of the theoretical maximum. The time taken to reach this value was noted for each compound.

Table II shows the values of the ED50 in mg./kg. of the five compounds, prepared in neutralized solution as described, kept at 30° for the time intervals shown, extracted with ether, kept on ice, and determined. The height of the pressor responses was measured in mm. and % inhibition of the mean initial response plotted against the log-dose of antagonist compound. The values of the ED50 were derived from these plots and were used to determine the points on the curves of Figs. 1 to 5.

Table II shows that antagonism to adrenaline was more marked than that against noradrenaline. Antagonism to histamine and to 5-HT was more variable, but usually 5-HT was less easily antagonized than noradrenaline and more easily antagonized than histamine. In some dogs small doses of the 2-haloalkylamine compounds potentiated the pressor response to injected adrenaline. Potentiation of histamine and 5-HT was more marked and more frequent, and it happened after much higher doses of compound. On occasions this led to a persistent increase rather than a decrease in the response to histamine and 5-HT; the phenomenon was never encountered with adrenaline or noradrenaline. In some dogs, particularly with compound AT2, no accurate determination of the ED50 against histamine and 5-HT could be made because potency was feeble. The times at which maximum amounts of ethyleneiminium ion were found in the kinetic studies were the times at which greatest biological activity was usually detected. Activity varied along the time scale in a manner similar to the variation in concentration of ion. This relation was more precise for antagonism to adrenaline and noradrenaline than to histamine and 5-HT. In the instance of 5-HT, lack of correlation may be due in part to the variation in dogs of the responses to repeated injections of standard amounts of amine.

The relation between the amount of titratable ethyleneiminium ion in solutions of these compounds and pharmacological activity is clearer if we plot ion production and activity at the various points in the time scale examined as % of the peak values obtained. These plots for the five compounds and the various amines are shown in Figs. 1 to 5.

Chloro-benzyl Derivatives.—The relation between the curves of production and decay of ethyleneiminium ion and potency as an antagonist of the pressor activity of adrenaline and nor-

TABLE II

MEDIAN EFFECTIVE DOSES OF NEUTRALIZED SOLUTIONS OF 2-HALOETHYLAMINES OF DIFFERENT AGE

The Table shows the relation between the time in min. for which a neutralized solution of 2-haloethylamine is kept at 30° and the ED50 in mg./kg. body weight in dogs anaesthetized with pentobarbitone sodium. The median effective dose was determined against (−)-adrenaline 4 μg./kg. (A), an equimolar amount of (−)-noradrenaline (N), or histamine (H), and five times as much 5-hydroxytryptamine (HT). The sign + indicates that the effect of the agonist was potentiated. An asterisk indicates the point in the time scale at which peak production of ion occurred as determined in kinetic studies, except in the case of compound AT_2, in which reactivity was so great initially that an arbitrary point at 10 min. was chosen.

N-Ethyl-N-chlorobenzyl-2-chloroethylamine Hydrochlorides

Time (min.)	JFA8			Time (min.)	JFA11			Time (min.)	JFA14			
	A	N	H		A	N	H		A	N	H	HT
1	1·90	4·00	5·86	1	0·85	0·93	2·95	1	1·97	4·17	14·5	1·43
10	0·71	1·26	2·80	10	0·13	0·21	0·39	10	0·28	0·50	+	+
*30	0·61	1·01	1·21	*30	0·11	0·17	0·50	*50	0·26	0·38	1·31	0·50
120	0·85	1·53	2·30	120	0·13	0·19	6·30	120	0·37	0·68	1·59	1·51
240	0·90	2 00	4·65	240	0·20	0·28	1·04	240	0·70	1·21	2·40	0·78

N-Ethyl-N-9-fluorenyl-2-haloethylamine Hydrohalides

Time (min)	AT_1				Time (min.)	AT_2			
	A	N	H	HT		A	N	H	HT
3	0·21	0·37	2·17	0·96	0·5	0·09	0·22	1·65	5·28
*15	0·073	0·16	2·0	0·25	*10	0·15	0·40	1·27	1·13
30	0·077	0·18	>3·0	5·4	30	0·27	0·66	+	>10
60	0·10	0·25	6·0	2·27	60	0·75	1·30	14·1	>10
120	0·18	0·44	16·7	2·18	120	3·0	4·0	9·8	>10
240	0·24	0·79	8·3	2·50					

adrenaline was particularly close with the *m*- and *p*-chlorobenzyl compounds (Figs. 1 and 2; compounds JFA8 and JFA11) and rather less so with the *o*-chloro compound (Fig. 3; compound JFA14). The relation became less close and the

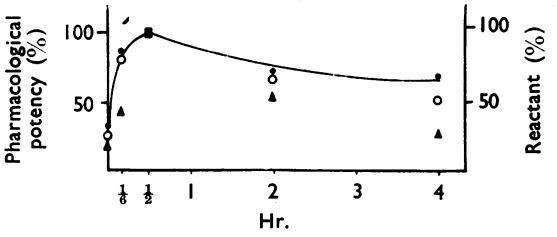


FIG. 1.—The relation between chemical reactivity and pharmacological activity in *N*-ethyl-*N*-*p*-chlorobenzyl-2-chloroethylamine HCl (JFA8). The line represents the variation with time of the production and decay of ethyleneiminium ion expressed as % of the maximum found at the point of peak activity (■, right-hand ordinate). The pharmacological potencies are expressed as % of the peak activities, measured as ED50 on the blood pressure of dogs anaesthetized with pentobarbitone sodium 30 mg./kg. (left-hand ordinate). ● represents the antagonism to (−)-adrenaline, 4 μg./kg.; ○ represents the antagonism to equimolar amounts of (−)-noradrenaline; ▲ gives the antagonism to equimolar histamine.

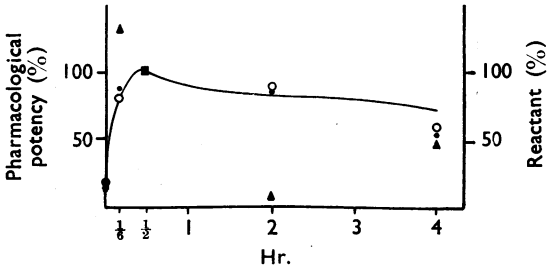


FIG. 2.—The relation between production of ethyleneiminium ion and antagonism to adrenaline (●), noradrenaline (○), and histamine (▲) in *N*-ethyl-*N*-*m*-chlorobenzyl-2-chloroethylamine HCl (JFA11). See Fig. 1 for explanation. There is no close correlation with antagonism to histamine.

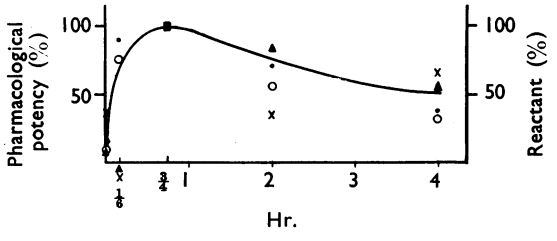


FIG. 3.—The relation between production of ethyleneiminium ion and antagonism to adrenaline (●), noradrenaline (○), histamine (▲), and 5 times equimolar 5-hydroxytryptamine by *N*-ethyl-*N*-*o*-chlorobenzyl-2-chloroethylamine HCl (JFA14). See Fig. 1 for explanation. The sign X represents antagonism to 5-HT. The responses to histamine and 5-HT were potentiated by all doses of the ionized solution aged 10 min. in 3 of the 5 dogs used, and are shown below the baseline.

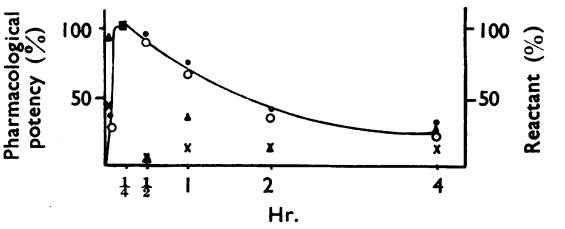


FIG. 4.—The relation between production of ethyleneiminium ion and antagonism to adrenaline (●), noradrenaline (○), histamine (▲) and 5-HT (X), by *N*-ethyl-*N*-9-fluorenyl-2-chloroethylamine HCl (AT1). See Fig. 1 for explanation. There is no correlation with antagonism to histamine or 5-HT.

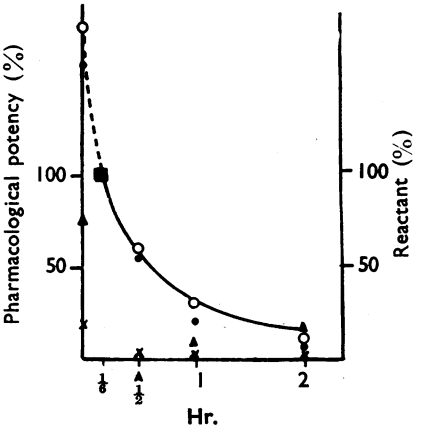


FIG. 5.—The relation between production of ethyleneiminium ion and antagonism to adrenaline (●), noradrenaline (○), histamine (▲), and 5-HT (X), by *N*-ethyl-*N*-9-fluorenyl-2-bromoethylamine HBr (AT2). See Fig. 1 for explanation. Due to the speed of reactivity, the levels measured after 10 min. reaction were expressed as 100%; the points on the curve of reactivity prior to that time were established less critically and are expressed by an interrupted line. They are not extrapolations but approximate measures. The correlation with antihistamine activity is poor; there is no correlation with the low level of activity against 5-HT shown by this compound.

scatter of individual values increased in solutions 4 hr. old. A causal relationship between the amount of ion in solution and antagonism to the hypotensive action of histamine was not established for the *m*-chlorobenzyl compound (Fig. 2; compound JFA11), but it may exist with the other two compounds. Likewise, the relation of ion production with antagonism to the pressor response to 5-HT (Fig. 3; compound JFA14) was not close although the general trend was for the two properties to move together.

9-Fluorenyl Derivatives.—The correspondence between ion production and decay with time and antagonism to adrenaline and noradrenaline was close for the chloro- and bromo-9-fluorenyl compounds AT1 and AT2 (Figs. 4 and 5), but again

was much less close for antagonism to histamine and 5-HT. There was no reduction in antagonism to adrenaline and noradrenaline relative to ion content of the solutions with ageing at 4 hr. as was seen with the chlorobenzyl compounds.

Activity of N-*Ethyl*-N-*Chlorobenzyl Ethyleneiminium Picrylsulphonates*

Sodium picrylsulphonate in doses up to 20 mg./kg. injected intravenously did not alter the basal blood pressure of the anaesthetized dog and had no effect on the response to repeated injections of adrenaline, noradrenaline, histamine, and 5-HT. In the spinal rat, the result was not quite so clear. It was impracticable to examine quantitatively the responses to adrenaline, noradrenaline and 5-HT on one such preparation because the administration of 5-HT modified the responses to the other two amines. Sodium picrylsulphonate in doses up to 32 mg./kg. did not alter the blood pressure but had a variable effect on the pressor responses to the amines, particularly 5-HT. In most rats, the responses to repeated injections of adrenaline and noradrenaline, or of 5-HT, were not altered by less than 1 mg./kg. of picrylsulphonate, which corresponded to a greater amount than was present in the ED50 of the ions P1 to P3. With amounts greater than 1 mg./kg. the responses were nearly always potentiated, which meant that the slopes of the dose response lines from which the ED50 values were derived tended to be less steep at higher dose levels of the antagonists and the ions thus appear to be less effective than they were. This source of error was never more than 15% of the initial response, was unpredictable, and its net result was to diminish rather than to exaggerate the difference in activity of a compound administered as salt of ion or as salt of parent compound; it was therefore ignored.

It was not possible to obtain solutions of the ions as picrylsulphonates which consumed more than 94% of the theoretically possible amount of thiosulphate, despite apparent analytical purity and constant melting point (Allen and Chapman). The solutions therefore contained only 94% of active ion credited to them. This factor also tended to make the ions appear to be less effective.

Fig. 6 depicts the effect of injections of a solution of the picrylsulphonate of one of these ions (P3) on the blood pressure of a dog anaesthetized with pentobarbitone sodium. Doses which reversed the pressor response to adrenaline had no effect on the resting blood pressure; larger doses, which abolished the pressor response to 5-HT and greatly diminished or abolished the response to noradrenaline, lowered the resting blood pressure only slightly. The effect on the response to histamine was slight and not related to the dose of compound administered. The general pattern of responses was very similar to that produced by the parent haloethylamine.

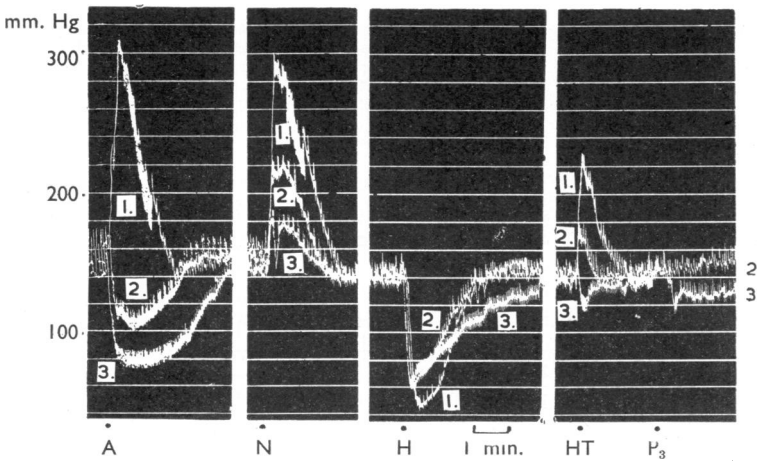


FIG. 6.—Dog, male, 11.3 kg., pentobarbitone sodium 30 mg./kg. i.v., atropine 3 mg. s.c. Record of the carotid blood pressure. The panels record the effect of (−)-adrenaline, 4 μg./kg. (A1), equimolar (−)-noradrenaline (N1), equimolar histamine (H1), and 5 times equimolar 5-HT (HT1). Two injections of 0.30 mg./kg. of *N*-ethyl-*N*-*o*-chlorobenzyl ethyleneiminium picrylsulphonate (P3), given at an interval of 30 min. (P_3 2 and 3) were allowed to act for 5 min. and the standard injections repeated. The basal pressure was slightly reduced (P_3 3), the response to adrenaline reversed (A2 and 3), that to noradrenaline reduced (N2 and 3), that to histamine slightly reduced (H2 and 3), that to 5-HT reduced or reversed (HT2 and 3).

The actions and potencies of the ethyleneiminium ions derived from the three chlorobenzyl compounds and of the parent compounds are recorded in Table III.

Table III reveals several points of interest. The three ions antagonized the changes in the blood pressure of the anaesthetized dog and spinal rat brought about by injection of adrenaline, noradrenaline, histamine, and 5-HT. There were species differences in these activities. The *p*-chlorobenzyl ion, P1, was less active than the *m*- and *o*-chlorobenzyl ions P2 and P3, and its parent compound JFA8 less active than the *m*- and *o*-chlorobenzyl compounds JFA11 and JFA14. There was not much difference in the potencies of P2 and P3 ions, but considerable

TABLE III

THE VALUES OF THE ED50 FOR THE ETHYLENEIMINIUM IONS AND FOR THE PARENT COMPOUNDS

The values of the ED50 mg./kg. of three ethyleneiminium ions and of parent compounds which antagonize the response of the blood pressure to adrenaline (A), noradrenaline (N), histamine (H), and 5-hydroxytryptamine (HT) in the spinal rat and the anaesthetized dog. The estimates are corrected in terms of % of ion available. (c) indicates that the antagonism was weak and the value of the ED50 was only approximate. The sign — indicates that the substance was not tested. The values for the ED50 of the parent compounds are expressed in terms of the maximum theoretical amount of ion available if the compound were fully ionized, and also in terms of the maximum actually found at the time of peak concentration of ion as determined by consumption of thiosulphate. The ratios of the values for the ED50 of the ions which were given as picrylsulphonates and of the ions which were available from the parent compounds are also shown.

	(1) Ion as Picrylsulphonate (P)							Available Ion Pure (1a)						
	Rat			Dog				Rat			Dog			
	A	N	HT	A	N	H	HT	A	N	HT	A	N	H	HT
P_1 ..	0·60	0·84	1·10	1·30	2·40	2·90	1·00	0·240	0·336	0·440	0·520	0·960	1·16	0·400
P_2 ..	0·42	0·57	0·98	0·22	0·37	5	0·19	0·168	0·228	0·392	0·088	0·148	2	0·076
P_3 ..	0·32	0·48	0·78	0·20	0·42	5	0·60	0·128	0·192	0·312	0 080	0·168	2	0·240
	(2) Parent Compound as Hydrochloride							Available Ion (Max. Theoretical) (2a)						
JFA8 ..	9·80	10·60	16·2	6·1	7·9	c 9·0	—	7·15	7·73	11·8	4·45	5·76	c 6·5	—
JFA11 ..	1·31	1·35	6·3	4·0	6·2	c 12	—	0·95	0·98	4·6	2·92	4·52	c 8·7	—
JFA14 ..	6·43	5·93	10·2	1·6	2·8	c 16	—	4·69	4·32	7·4	1·16	2·04	c 11·6	—
	Approx. Ratio ED50. $\frac{\text{Ion Found from Parent}}{\text{Pure Ion (P)}} = \frac{2b}{1a}$							Available Ion (Max. Found) (2b)						
JFA8/P1	10	9	10	3	2	2		2·570	2·780	4·25	1·60	2·07	2·34	—
JFA11/P2	1·5	1·1	3	8	7	1·13		0·247	0·255	1·19	0·76	1·17	2·26	—
JFA14/P3	8	5	5	3	2·6	1·25		1·030	0·950	1·62	0·25	0·44	2·50	—

difference in the potencies of their parent compounds JFA11 and JFA14, particularly in the rat. The number of animals used in each test (5 rats and 5 dogs) seemed adequate, and sufficient time was allowed for the parent compound to exert maximum effect before testing. The antagonism of the ions and their parent compounds to adrenaline was more marked than their antagonism to noradrenaline. The pattern of antagonism to histamine and 5-HT was less consistent. In the rat, 5-HT was antagonized less than noradrenaline by the ions and their parent compounds; in the dog 5-HT was antagonized more than histamine by the ions. The parent compounds were not active antagonists of histamine; 5-HT was not tested. Potentiation effects were not so marked as with the treated solutions of parent compounds (see Table II) and did not prevent the development of inhibition of the responses to histamine and 5-HT. Such effects were, however, present in some animals with some doses of either ion or parent compound in relation to any one of the agonists. The ED50 of the ion, expressed either as weight/kg. of its picrylsulphonate or as available ion, was always less than the ED50 of the parent compound, expressed as weight of halogen hydracid salt, or as available ion in terms of the theoretically possible maximum, or in terms of % found *in vitro* by consumption of thiosulphate. For both species of test animal and all 4 agonists the ratio of these active amounts (ED50) was in all cases greater than unity, but there was a wide scatter in the indices.

DISCUSSION

The evidence provided in Table II and the graphic presentation in Figs. 1 to 5 make it clear that a quantitative relationship exists between the anti-adrenaline and anti-noradrenaline activity of these 2-haloethylamine compounds and the amount of ethyleneiminium ion present in the solution. With antihistamine activity, the relationship is reasonably good for two of the chlorobenzyl molecules and poor for the other; there is no such relationship for the two 9-fluorenyl compounds. With anti-5-HT activity, the relationship is discernible with the only chlorobenzyl compound examined but not with the 9-fluorenyl compounds.

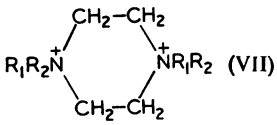
We may therefore conclude with some degree of confidence that over a wide range of structure (three *N*-chloro-benzyl haloethylamines and two *N*-9-fluorenyl-haloethylamines reported above, and three *N*-naphthylmethyl haloethylamines examined similarly by Graham and Lewis, 1954) there is a direct proportionality between the antagonism to adrenaline and to noradrenaline and the concentration of thiosulphate-consuming species and a limited relationship to antihistamine and anti-5-HT activity. The latter relationship varies with the structure of the compound examined. In addition

there is the evidence provided by Chapman and James (1953) and Graham and Lewis (1953) in kinetic and pharmacological studies that, if the halogen in the alkyl chain is fluorine, compounds are non-reactive chemically and inactive pharmacologically, whereas the substitution of any other halogen for fluorine restores both properties to a similar degree in these molecules.

If the active haloethylamines are dissolved in an aqueous medium, the base may be liberated from the salt and left for an appropriate time. The unchanged parent compound may then be extracted from the mixture by appropriate treatment with ether, and the residue still contains a thiosulphate-consuming species. The formation of acid can be followed during the whole of the reaction, particularly in its early stages. With a compound which liberates the whole of its halogen immediately and gives a corresponding amount of ethyleneiminium ion there is scarcely any initial acidity. This was shown by Chapman *et al.* (1952) and Chapman and James (1954). The intermediate formed is therefore likely to be an ethyleneiminium ion and not a vinylamine.

The drift away from a close correlation between anti-adrenaline and anti-noradrenaline activity and the curve of decay of ion in initially neutral solutions of compounds JFA8 and JFA14, and to a lesser extent JFA11, has no theoretical explanation but may be due to the fact that neutralized solutions of the ages involved (4 hr.) were relatively inactive and usually required a greater number of injections in order to establish a satisfactory dose/response relationship. The experimental design of the work was such that the three solutions of age 4 hr. were investigated before it was realized that decay of the ion in neutral aqueous solution would take place to an appreciable extent at room temperature if there was delay beyond 60 min. in establishing a result. The potencies at these points could probably be raised by 10% with justification, but the correction has not been made because there is no precise record of the time for which the samples were kept before injection. All subsequent work was done with the aqueous solutions kept on ice. No such discrepancy was observed by Graham and Lewis (1954) with the naphthylmethyl compounds and the phenomenon is probably devoid of true significance.

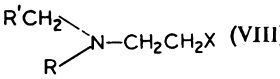
The picrylsulphonates of ethyleneiminium ions were prepared by precipitation at 0° by acid sodium picrylsulphonate of a neutralized solution in acetone water of the 2-haloethylamine salt concerned (Chapman and James, 1954; Allen, 1956), and ultimately analysed. It has been suggested that the dimer or piperazinium ion (VII),



(VII)

would give rise to the same ultimate analysis as the ethyleneiminium ion and might be the active species. There are two points of evidence against this. (1) The supposed ethyleneiminium ions (compounds P1 to P3) consume thiosulphate to an extent of 94% of the theoretical maximum. Simple piperazinium compounds do not consume thiosulphate; piperazinium ions from active 2-haloalkylamines have not been tested. (2) The piperazinium salts from certain active 2-haloalkylamines (including compound *N*-ethyl-*N*-naphthylmethyl-2-chloroethylamine HCl [J10 in the list of compounds examined by Graham and Lewis, 1953, 1954] have been made, tested, and reported inactive by Nickerson and Gump (1949). The picrylsulphonate compounds P1 to P3 are very active and the compound J10 is active.

Chapman *et al.* (1952) have shown that for the series of naphthylmethyl-2-haloethylamine derivatives (VIII) where R=phenyl, R′=1- or 2-naphthylmethyl and X=Cl, Br or I, there



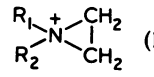
(VIII)

is no consumption of thiosulphate, very little liberation of halide ion, and no formation of ethyleneiminium ion. Graham and Lewis (1953) found that these compounds were inactive. Ing (1956) has pointed out that the phenyl group attached directly to the *N* atom reduces the basicity of the amine sufficiently to prevent intramolecular alkylation (see Chapman and James, 1953).

Peart (1956) has objected that the evidence of Harvey and Nickerson (1953) as to the relation of chemical transformation of dibenamine and dibenzyline to biological activity in these compounds is only indirect. A biologically inert β-haloalkylamine (*N*:*N* - dicyclohexyl - β - chloroethylamine) gave the same indirect chemical reactions (evolution of Cl^- and H^+ and consumption of thiosulphate) which are supposed to indicate ethyleneiminium ion formation. He continues: "The fact that thiosulphate could prevent the block if it was administered before, or simultaneously with, the β-haloalkylamine was used as evidence that such an intermediate was formed, since it was known

that thiosulphate reacts with similar derivatives of nitrogen mustards. However, it will also probably react directly with unchanged β-haloalkylamine so that overall the evidence in favour of an active intermediate is inconclusive."

As to the first point, it has never been suggested that all structures of the ethyleneiminium type are active anti-adrenaline compounds, though all active 2-haloethylamines are alkylating agents. Much depends on the conditions to be fulfilled by the substituent groups R_1 and R_2 in the ion (IX).


(IX)

All active anti-adrenaline compounds of the dibenamine type are necessarily alkylating agents, but it does not follow that all alkylating agents are anti-adrenaline compounds. The importance of the substituent on the nitrogen atom is illustrated in the results of the present investigation. The order of potency of the *N*-ethyl-*N*-chlorobenzyl-2-chloroethylamine HCl compounds is found to be JFA11>JFA14>JFA8 in the spinal rat, which technically is the better preparation in which to measure antagonism to pressor substances. The order of availability of ion *in vitro* from these compounds is JFA8>JFA11>JFA14. The order of potency in the same animal preparation of the ions derived from these compounds is ion of JFA14>ion of JFA11>ion of JFA8. In addition, in all cases the injection of the ion as a picrylsulphonate into the blood stream proves to be more potent than the injection of the same available amount of ion when it has to be derived by cyclization from its parent haloethylamine and the indices of relative activity vary with species and agonist (Table III). It follows that the factors controlling solubility and reactivity of the parent compound *in vivo* may be as important to its effectiveness after administration as the potency of the pure ion. The haloethylamine which reacts to the greatest extent in neutral solution (JFA8) is the least potent because the effects of the group R^2 are such as to give least potency as an antagonist; the haloethylamine with the most potent ion structurally (JFA14) cyclizes to the least extent and is therefore of only moderate potency. The most potent is such that it cyclizes well and produces an effective ion. The rate of ionization in buffered protein-containing medium is obviously of great importance.

As to the second point discussed by Peart (1956), it is likely that thiosulphate will react with parent halogenoalkylamine, but this is difficult to prove because it is always possible for the parent substance to form the ethyleneiminium ion and react by that route. If the halogen is inert, as in fluorine compounds, no thiosulphate is consumed, and there is no biological activity.

The evidence provided in this paper in conjunction with the investigations of Chapman and his colleagues on kinetics show that antagonism to adrenaline and noradrenaline is dependent on production of ethyleneiminium ion at an effective rate, in adequate amounts, and of a proper structure. It does not throw light on the relationship between structure, ion formation, lipoid solubility and duration of effectiveness discussed by Nickerson (1949), Hunt (1949), and Axelrod, Aronow, and Brodie (1952), but makes it clear that these criteria must be observed before the other matter can be properly discussed. Dibenamine is not the best substance for such studies.

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