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THE INFLUENCE OF ONIUM-SUBSTITUTION ON THE NEUROMUSCULAR BLOCKING PROPERTIES OF SOME QUATERNARY AMMONIUM COMPOUNDS.

The author wishes to acknowledge his indebtedness to Professor J.B. Stenlake for suggesting the problem and for his stimulating direction throughout, Professor J.P. Todd for his help and encouragement and Drs. Comrie and Williams for their interest and ever-willing advice, and also the Pharmaceutical Society for the award of an educational grant.

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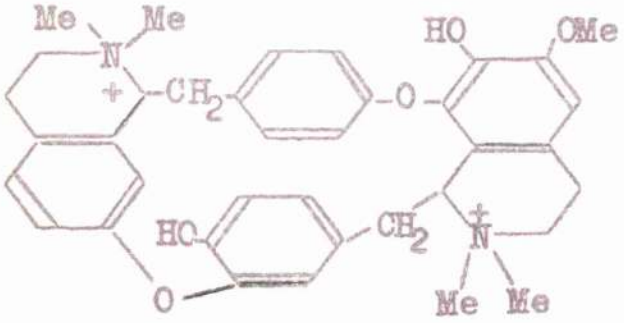
EXPERIMENTAL .

INTRODUCTION

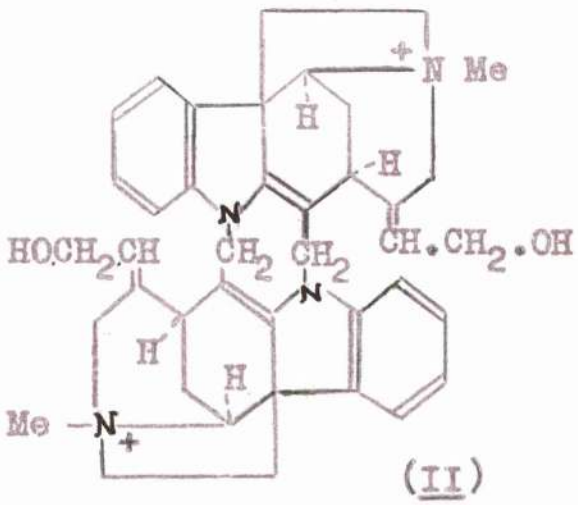
HISTORICAL .

The term "Curare" in its modern sense refers to concentrated plant extracts which produce a paralysis of skeletal muscle due to the blockade of acetylcholine transmission at the neuromuscular junction. The extracts are prepared by South American natives from a botanical source which is generally confined to certain members of the families Loganiaceae and Menispermaceae, in particular, Strychnos toxifera and Chondodendron tomentosum respectively.

The active chemical principles are tertiary and quaternary alkaloids of complex structure which can be divided into two distinct types depending on their botanical origin. Those from Chondodendron species have a bisbenzyl-tetrahydroisoquinoline structure similar to that of (+)-tubocurarine (I), while those from Strychnos species have a bisindolic structure similar to toxiferine I (II).



(I)



(II)

The history of curare is so interwoven with that of other obscure South American arrow poisons that it may be presumed to date back to the Spanish conquest. Among the earliest references to the action and preparation

of these arrow poisons are those of d'Anghera between 1516 and 1530, in his chronicles of the new world.(1) Whether the name was first introduced by Sir Walter Raleigh(2) or Keymis(3) in 1595 as "ourari" or by Margraaf in 1648(4) as "curuiri," is uncertain, but by the eighteenth century the word "curare", spelled in a variety of ways, was in current use. By the second half of the eighteenth century curare had become the main arrow poison of interest to the explorers and botanists, and efforts were made towards finding the botanical source of the "true" curare. The very important role of the Loganiaceae and Menispermaceae became evident from the work of such people as von Humboldt, von Martius, the Schomburgks, Castelnu and many others during the nineteenth century.(5) In general curares from the forest regions of Equador and Peru contain ingredients derived from several varieties of Chondodendron, whereas those from the more easterly curare producing regions in the Guianas and lower Orinoco contain compounds from Strychnos species. Other curares may contain both, and all may contain other ingredients to increase the consistency of the product or aid its absorption.(6)

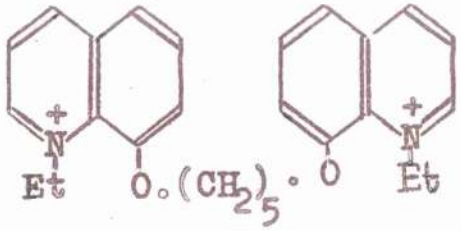
The first carefully conducted experiments with curare were published by Fontana in 1781(7) in his "Treatise on Poisons". Magendie's use of curare to immobilise his test animals, before the advent of chloroform and ether as

anaesthetics, led to Claude Bernard's interest in its mode of action.[8,10] Bernard's experiments[9] established that the action of curare was localised to some point between nerve and muscle. This finding led to the discovery of the nerve end-plates and muscle sole-plates which, in turn, paved the way for our present knowledge of chemical transmission at the neuromuscular junction.[10]

The early chemistry of curare was hampered by the lack of any adequate classification of the various curare preparations. Thus Boehm's[11] findings that these preparations could be distinguished by their containers was of great importance and gave rise to the terms, "tube", "pot", and "calabash" curares. Boehm also showed that the alkaloids consisted of amorphous quaternary bases which were physiologically active, and tertiary bases which were inactive.[12] However, it was not until 1935 that King isolated (+)-tubocurarine, the first pure, crystalline quaternary alkaloid, and elucidated its structure.[13] The first isolation of crystalline alkaloids from "calabash" curare was achieved by Wieland and co-workers in the period 1937 to 1941.[14] Although these alkaloids were shown to differ chemically from (+)-tubocurarine, Paton found that their action at the neuromuscular junction was very similar.[15] This work has now been greatly extended by Karrer and Schmid and Battersby and Hodson and their colleagues, and is the subject of an excellent review article by the latter workers.[16]

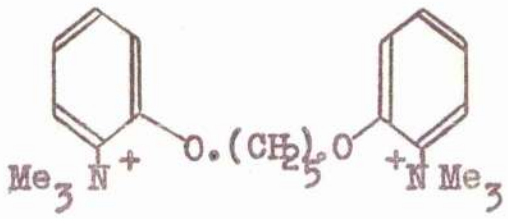
The isolation and very high potency of (+)-tubocurarine renewed medical interest in curare and the work of Wintersteiner and Dutcher,[17] and Holaday[18] led to the introduction of "Intocostrin", the first biologically standardised curare preparation. Such was its success that within ten years the use of curare-like agents as adjuncts in anaesthesia had become standard practice in most operating theatres. The advantage of these agents is that adequate muscle relaxation can be obtained during surgery without a profound, central narcosis and its accompanying disadvantages.[19] The untoward side effects of "Intocostrin", such as a paralysis of autonomic ganglia and histamine release, together with the high cost and rather uncertain supply of curare led to the development of the synthetic drugs.

Although it was known from the time of Crum-Brown and Fraser[20] that curare-like activity was a property of quaternary ammonium salts, the significance of the bisonium structure was first demonstrated by Bovet in 1946 with his synthesis of 1,5-pentamethylenebis(1'-ethyl-8'-oxyquinolinium) di-iodide (III).



(III)

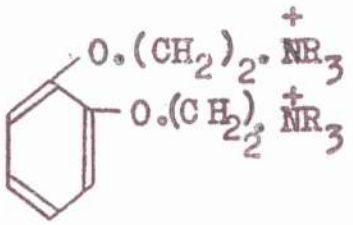
This compound had an activity and selectivity of action comparable to that of (+)-tubocurarine,[21] and was used clinically for a time. Further work by Bovet and his colleagues on phenolic ethers,[22] such as compound (IV),



2 I'

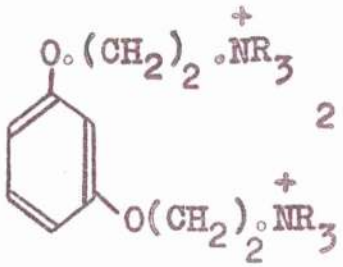
(IV)

and ethers formed from choline, or homologous aminoalcohols, and various phenols,[23] led to the development of the compounds (V), (VI), (VII), and (VIII), where R is a methyl or ethyl radical.



2 I'

(V)



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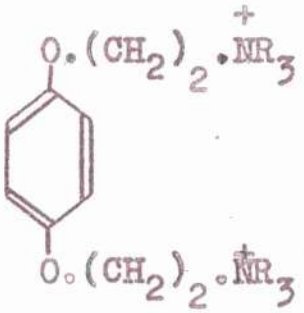

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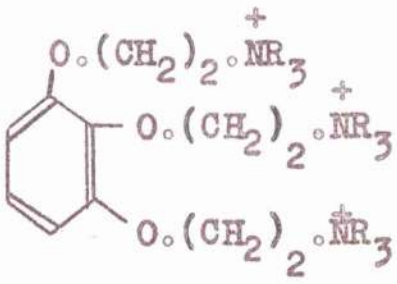


3 I'

(VIII)

Gallamine triethiodide, the trisonium salt (VIII, $R_3 = Et_3$) is less active than (+)-tubocurarine but has fewer side effects. Shortly afterwards the simultaneous publication by Barlow and Ing, and Paton and Zaimis[24] of the potency of linear polymethylenebistrimethylammonium salts showed conclusively the importance of having two onium groups at an optimum distance apart. These workers found that the potency of their compounds varied considerably with the test preparation but that the decamethylene compound, decamethonium iodide (IX), was two to three times as active as (+)-tubocurarine on the rabbit head drop test.

$$Me_3\overset{+}{N}.(CH_2)_{10}.\overset{+}{N}Me_3 \quad 2\,I^-$$

(IX)

On this preparation potency increased gradually from the ethylene to the nonamethylene member, reached a sharp maximum with decamethonium and fell off thereafter. Paton and Zaimis also indicated that the block produced by decamethonium was antagonised by (+)-tubocurarine. These findings provided the basis for almost all subsequent research on synthetic neuromuscular blocking agents.

The Receptor Theory and the Neuromuscular Junction.

Neuromuscular blocking agents are considered to be structurally specific drugs and, as such, exert their action by interacting with acetylcholine receptors at the neuromuscular synapse preventing the transmitter from exerting its normal physiological response. The molecular complement theory utilises both stimulating and blocking drugs to elucidate the structure of the receptors upon which they act on the assumption that the molecular dimensions and physical properties of the receptor will be complementary to those of the drugs. Cholinergic receptors and the active surface of the cholinesterase enzyme have been studied in this way.(25) Where flexible molecules are used statistical studies, such as those employed by Gill(26) for ganglion blocking agents, can be employed to determine their most probable conformations. The specificity of cholinergic blocking agents suggests that there are at least three types of cholinergic receptor which may differ chemically or in their arrangement on the synaptic membrane. Since the ganglia differ from the neuromuscular junction anatomically by possessing a membrane sheath, other factors such as membrane permeability, lipoid solubility and affinity of the drugs for non-specific receptors might also be implicated. The recent discovery, however, that the acetylcholine ion exists in two different conformations in the crystal

lattice[27] would be expected to have a biological significance. In addition to the usual extended conformation the molecule also exists in a "ring" form in which the methyl group of the choline radical approaches to within 3 A° of the ester oxygen atom. (X)

Acetylcholine ion

Extended form "Ring" form

(bond distances in Angstrom units)

(X)

Cavallito has suggested that the ring forms, (XI)

(XI)

would be energetically most stable in solution.[28] Infra-red spectrophotometric studies by Fellman and Fujita[29] suggest that in ethanolic solution acetylcholine does exist in a ring form.

The idea of a "receptive" substance in the muscle which is responsible for transmitting nerve impulses was introduced by Langley[30] to account for the action of curare, but it is only recently that direct experimental evidence for its existence has been provided.[31] It has been suggested that the receptor is a protein component of an enzyme system.[32] Direct attempts to isolate the receptor have been made by Chagas[31,d] and by Ehrenpreis,[31,e] using the electroplaque of the eel Electrophorus electricus, and the latter has obtained a homologous protein extract with striking receptor-like binding properties. Although the evidence of a receptor appears certain several questions remain unanswered, such as its chemical structure, the nature of its reaction with acetylcholine and the receptor arrangement on the membrane.

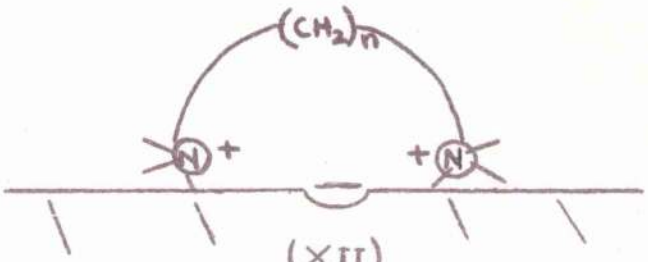
The very general curare-like action of onium salts at once suggests that the receptor has an anionic site which forms electro static bonds with the onium cation. The reversibility of the reaction would also tend to rule out covalent bonding. Ing and Wright[34] stated that the most probable reaction would be some ionic exchange or permutit-like reaction in which the onium ion replaces some normal cation in the structure upon which the drug acts. Some workers believe that a molecule such as acetylcholine has only a limited number of ways in which to react with a

protein. Thus information obtained from the reactions of cholinesterase will provide a better understanding of those between acetylcholine and the receptor.[33]

Waser[25,d] and Standaert and Friess[25,i] have concluded that the receptor must have a definite three dimensional structure and that cholinergic molecules must fit into it. Nachmansohn[33,a] also believes that the quarternary head of acetylcholine must be enveloped by the receptor, thus producing a change in configuration which allows the passage of sodium and potassium ions. While Cavanaugh and Haaron[34] have found that for the initial rate of contraction of skeletal muscle a reaction in which two molecules of acetylcholine complex with each receptor molecule is kinetically favourable.

The significant increase in selectivity and potency of bisonium over mono-onium compounds is generally accepted as being due to bivalent bonding with the receptor. However, important effects on the physical properties of the molecule may account for the beneficial influence of the second onium group. Thus the altered hydrophilic-lipophilic ratio of the bisoniums may favour the concentration of the drug reaching the receptor by decreasing the loss at inactive sites.[35] Also, the ready formation of ion-pairs between a di-cation and a single anion (XII) as shown by conductimetric studies,[36] leads Cavallito to suggest[28] that a bisonium ion could form a much more stable bond with an anionic site than could

a mono-onium; but it is doubtful if this type of bonding will occur with the longer chain compounds since the "quasi" - ring structures would be unstable.



(XII)

It is very difficult to rationalise the high activity of bisonium salts having very different inter-onium distances with the concept of a single receptor structure. Although Gill[26] has suggested that the two anionic sites of the receptor may not be rigidly fixed, it would seem probable that more than one site of action is involved. Depolarising substances may displace acetylcholine from its protein storage complex[28] and this acetylcholine would then be responsible for the contracture producing properties of these compounds, while the non-competitive blocking agents of Ariëns[49] are presumed to block transmission by acting on other than acetylcholine receptors. Koelle[38] believes that, apart from the accepted post-synaptic receptors on the muscle sole-plate, acetylcholine also acts on pre-synaptic receptors on the nerve end-plate to release further quanta of acetylcholine, and these receptors may well differ from each other. A pre-synaptic site of action has also been attributed to (+)-tubocurarine.[39]

The activity of compounds of varied chain length can also be explained by some form of lattice arrangement of anionic sites on the synaptic membrane.[40,28,27]

The Classification of Neuromuscular Blocking Agents.

The transmission of nerve impulses at the neuromuscular junction can be blocked or otherwise affected by chemical agents in several ways.[41] These include interference with the production or release of acetylcholine as, for example, with the block produced by the hemicholiniums. Secondly, interference with the hydrolysis of acetylcholine by the cholinesterase antagonists eserine, neostigmine and tensilon also causes blockade due to the accumulation of excessive amounts of acetylcholine. A third group causes alteration of the end-plate sensitivity to acetylcholine at the neuromuscular junction mainly by a competitive inhibition. and can be very broadly divided into two types; the competitive or curarimimetic agents such as (+)-tubocurarine produce a blockade without depolarising the synaptic membrane, and their action is reversed by the anticholinesterase agents. While the depolarising or cholinomimetic agents, such as decamethonium and succinylcholine, depolarise the membrane before producing a blockade which is potentiated by anticholinesterases. All the principal features of a block by decamethonium or succinylcholine can be reproduced by acetylcholine in the presence of an anticholinesterase agent.[43]

The mode of action of competitive agents is both qualitatively and quantitatively more uniform in that there is less species variation than is seen among the depolarising drugs.[41] The difference between the two types is most marked in frog and avian muscle.[33,b] In mammals depolarising agents frequently show a mixed action and a "pure" cholinomimetic action is shown by decamethonium and succinylcholine only in the cat and man.[43]

The order of species sensitivity to (+)-tubocurarine is rat > mouse > rabbit > man = cat and the order is reversed for decamethonium.[44] Variations in sensitivity to blocking agents of different muscles of the same species has been extensively studied in the cat. Jewel and Zaimis[45] have shown that while decamethonium and succinylcholine produce a typical depolarisation block in the cat tibialis muscle, they cause a "dual" block in the soleus muscle. The dual block exhibits features characteristic of both types of block, as well as others, such as a striking decrease in sensitivity to repeated doses, which are entirely absent from both. This type of block is also found in the monkey, dog, rabbit and hare.[43]

The quantitative theory and mathematical treatment of the mode of action of drugs developed by Clark[46] has been modified and extended by Ariëns[47] and Paton.[48] These theories can account for the observed differences

between the "pure" and "mixed" types of action exhibited by neuromuscular blocking agents without inferring different modes of action for them. Ariëns and his colleagues have introduced the terms "affinity" and "intrinsic activity" as essential factors determining the type of action of any drug. Their theory also requires the existence of spare receptors and more than one type of receptor at the neuromuscular junction. The affinity is a specific constant for each drug which determines how much of the drug-receptor complex will be formed, while the intrinsic activity determines the physiological effect per unit of drug-receptor complex. On the basis of their chemical and pharmacological properties Ariëns believes that neuromuscular blocking agents have to be divided into at least three basic types. These are the depolarisers which have affinity to the acetylcholine receptors in the neuromuscular junction and which, like acetylcholine, have a high intrinsic activity. Then there are the competitors which have affinity towards the same receptors as the depolarisers but which have a very low intrinsic activity. Finally, there are the non-competitors which have affinity towards non-acetylcholine receptors and which are non-competitive antagonists of acetylcholine.(49) The features of the three types can be readily shown on cat or avian nerve-muscle preparations and are in good agreement with those predicted from the value of affinity and intrinsic

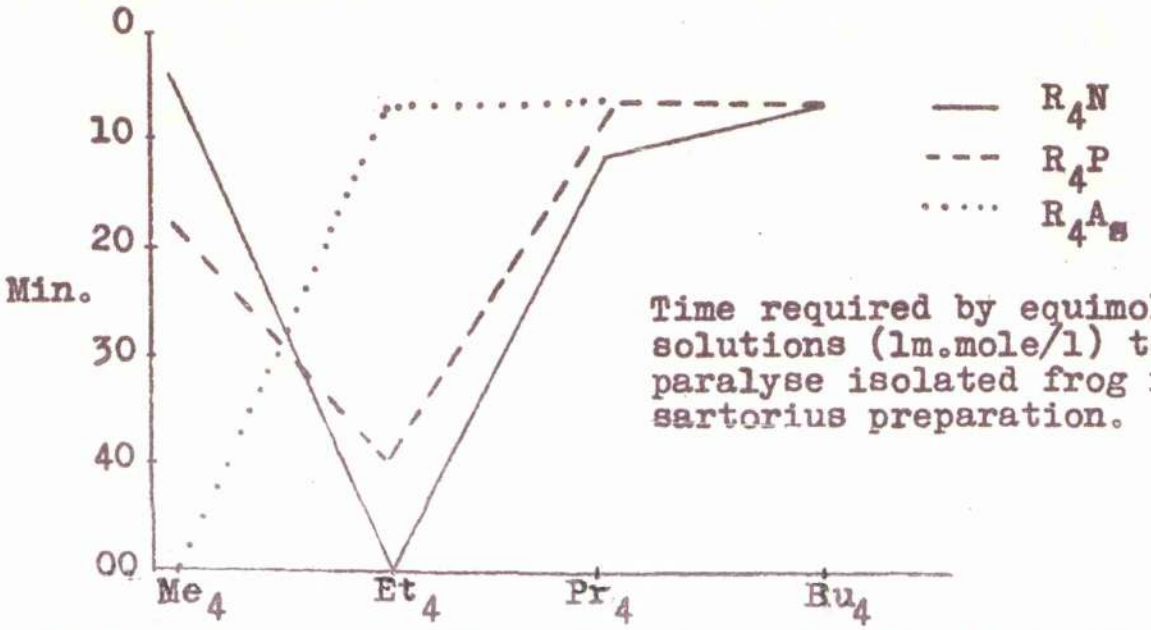
activity obtained on the isolated frog rectus abdominis muscle.[50] The depolarisers induce a contracture on chicken muscle and on cat muscle a flaccid paralysis which is not reversible by tensilon. The competitors produce a flaccid paralysis both in chick and cat muscles, which is reversible by tensilon, while the non-competitors produce a flaccid paralysis both in chick and cat muscle, which is not, or only to a small degree, reversible by tensilon. Simple changes in the chemical structure can produce a gradual change from one type of agent to another via intermediates which exhibit a mixed action. The intrinsic activity and affinity can vary independently with the chemical structure, and it does not appear possible to predict the optimal requirements for good affinity. The length and nature of the chain can both affect the intrinsic activity of bis-onium compounds. With regard to affinity towards non-competitive receptors, surface activity and lipoid solubility appear to be important.

MONO-ONIUM SALTS .

The neuromuscular blocking properties of onium salts were discovered in 1869 by Crum-Brown and Fraser who found that the methiodides and ethiodides of the alkaloids, strychnine, brucine, thebaine, codeine, morphine, atropine, and N-methylconiine, all possessed a curare-like action when tested on rabbits, dogs, cats, and frogs.[20] Many complex monoquaternary salts have been synthesised and tested since that time,[51,12] some of the more recent ones being based on the actual or supposed structures of (+)-tubocurarine and the "calabash" - curare alkaloids respectively.[52] Few systematic studies of onium substitution within particular series of these compounds have been performed, although the studies by Ing and Wright[53] are of note. Examination of the methiodides and ethiodides of strychnine, pyridine and tetrahydropyridine, and quinoline and tetrahydroquinoline led them to suggest that, when the nitrogen atom forms part of a ring system, replacement of a methyl by an ethyl radical in the quaternary salt increases the activity when the ring is aromatic, but decreases it if the ring is saturated.

Structure-action relationships have been more extensively examined among the simpler onium salts. The influence of alkyl substituents on the blocking activity of tetra-n-alkylonium salts has been studied on the frog sartorius muscle preparation.[53] Potency varied according

to the nature of the central onium ion, as shown in the graph. Thus, as the size of the central atom is increased, activity decreases for the tetramethyl, but increases for the tetraethyl derivative.



Time required by equimolar solutions (1m.mole/l) to paralyse isolated frog nerve sartorius preparation.

On the same preparation the order of activity in the n-alkyltrimethylammonium series was,[54] butyl- = amyl- = hexyl- = heptyl- = octyl- > propyl- > ethyl- > dodecyl-trimethyl ammonium. Similarly on the cat sciatic nerve-anterior tibialis muscle preparation[55] maximum activity resided in the butyltrimethylammonium salt, but activity decreased with increasing alkyl chain length, and was negligible in the tetradecyl derivative. The hexyl and lower homologues had a depolarising action and were mutual antagonists of (+)-tubocurarine, while the higher homologues did not antagonise (+)-tubocurarine, but had a more complex action.[56] Studies of the intrinsic activity of the compounds of this series, on the isolated

frog rectus muscle, showed that increasing the alkyl chain length brought about a gradual change from a depolarising to a non-competitive action.(50)

Marshall(57) found that the replacement of methyl groups by ethyl in tetramethylammonium chloride progressively decreased the activity on the isolated frog sartorius nerve-gastrocnemius muscle preparation. Similar results were obtained by Ing and Wright(54) who also observed that the introduction of two ethyl groups altered the characteristics of the block by producing a muscle twitch. Rossum and Ariëns have found that butylmethyl-diethylammonium and methyltriethylammonium also have stimulant properties.(50) According to Stovner(58) the low activity of tetraethylammonium is due to its ability to release acetylcholine from the nerve end-plate. Therefore it would seem probable that the above findings can be explained by a similar mechanism. In the n-alkyltriethyl-ammonium series affinity for non-competitive receptors appears with the n-propyl and steadily increases to the n-decyl derivative.(50) There is a positive correlation between non-competitive affinity and surface activity. However, lipoid solubility is also related to alkyl chain length and it is therefore not possible to decide whether surface activity or lipoid solubility is responsible for this affinity.

Charge Density Theory .

The importance of the density of the positive charge on the central onium ion in determining the level of activity of the simple monoquaternary salts was suggested by Holmes, Jenden, and Taylor.[59] This theory is based on Pauling's hypothesis,[60] that the unit positive charge on the ammonium ion is distributed approximately equally among the five atoms, and that in an onium ion, $[R_3X - R]^+$ the charge distribution will depend on the relative electronegativity of R and the onium atom X. These authors argue that the use of the dipole moments,

N ---> H	S ---> H	P ---> H	As <--- H
1.31×10^{18}D	0.68×10^{18}D	0.36×10^{18}D	0.10×10^{18}D

is valid in assessing the charge distribution across the corresponding, N - C, S - C, P - C, and As - C bonds. Thus the order of curare-like activity of the ions $Me_4N^+ > Me_3S^+ > Me_4P^+ > Me_4As^+$ parallels the charge density on the onium ion. Since conjugated systems have a charge distributing effect the theory can also explain the findings of Ing and Wright[53] that 1,1-dimethyltetrahydroquinolinium iodide and 1,1-ethylmethyltetrahydroquinolinium iodide are more active than the corresponding 1-methyl- and 1-ethyl-quinolinium iodides. And although the charge density theory by itself cannot explain why the pyridinium and quinolinium ethiodides are more potent than the

methiodides, this fact can be accounted for by assuming a hyperconjugation effect.[61,59] The methyl radical will hyperconjugate more readily than the ethyl causing an electron shift towards the ring which will decrease the positive charge on the onium nitrogen. The charge concentration on the onium ion is also regarded as an important factor for the bisonium compounds, especially those with a depolarising action.[50] However, the high activity of bisquaternaries is also largely dependent upon the chain length and the steric environment of the onium ion.

BISONIUM SALTS.

These compounds are too numerous to consider each of the many types. This discussion is therefore restricted to a consideration of onium substitution on certain representative examples which will be classified by the nature of the inter-onium chain. This structure may be an unsubstituted polymethylene chain, as in the methonium compounds, or it may contain ester, ether or amide groups, or various ring structures, all of which may influence potency and type of action.

A. Polymethylene Compounds.

(i) Alkyl Substituents

The stepwise substitution of methyl by ethyl radicals in decamethonium produces a considerable, but irregular reduction in activity on the rat diaphragm and the rabbit quadriceps preparations.[62] Activity falls to its lowest level with the introduction of the first ethyl group, but increases slightly with further substitution. However, in the hexa- and hepta-methylene members of the series (IX, n = 6,7) ethyl substituents are favourable for activity.[63] Thus there is a steady increase in potency with the successive replacement of the methyl radicals of heptamethylenebis (trimethylammonium)di-iodide which has only one-fifth the activity of heptamethylenebis (triethylammonium) iodide on the rat phrenic nerve diaphragm preparation. Barlow and Ing[64] examining an extensive series of polymethylenebis-trimethyl-(IX, $R_3 = Me_3$, n = 2-5, 7-13) and triethyl-ammonium bromides(IX, $R_3 = Et_3$, n = 2-10, 13), have found that whereas activity reached a sudden maximum on the rat phrenic nerve diaphragm test when n = 10 in the methonium series, there is a steady rise in activity from n = 4 to 13 in the ethonium analogues. Tridecamethylenebis (triethylammonium)di-bromide has about two-fifths of the potency of (+)-tubocurarine by the rabbit head drop test.

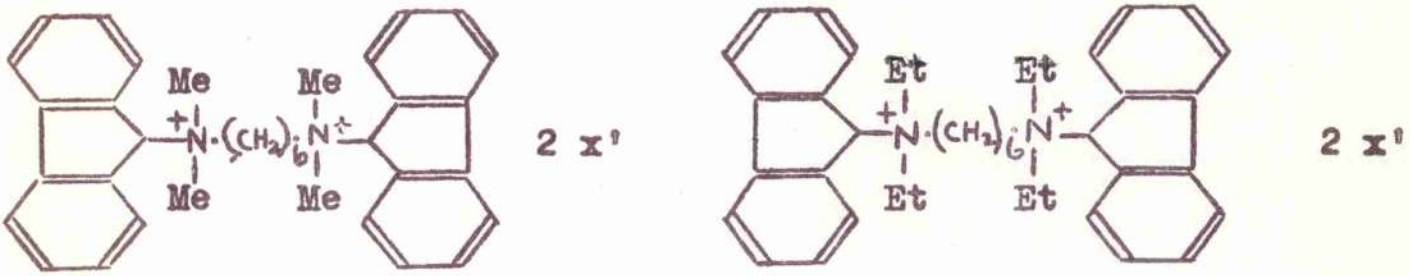
A precise study of the changes produced in the mode of action by alkyl substitution within a homologous series has been performed by Thesleff and Unna.[65] They used as their test preparations the sciatic nerve-gastrocnemius muscle preparations of the chicken and mouse, since the chicken is good for differentiating the type of block and is highly sensitive to depolarising agents[66] while the mouse is very sensitive to competitive agents.[67] In the methonium series of compounds, $\underline{n}$ = 2 to 10, all but penta- and hexa-methonium had a depolarising action, although tetra-, hepta- and octa-methonium also exhibited competitive properties, such as reversibility to anti-cholinesterases. The complete replacement of methyl by ethyl substituents in hepta- and deca-methonium completely abolished, or considerably decreased their depolarising properties, and rendered the paralysis reversible by tensilon or eserine; whilst similar replacement of methyl by ethyl substituents in penta- and hexa-methonium increased the potency, but did not alter the nature of the block. The mode of action of decamethonium was unaffected by the introduction of a single ethyl, isopropyl or butyl radical, but the tributyl-ammonium analogue of decamethonium showed the characteristic features of a non-competitive blocking agent.[50]

In a more extensive series of decamethylenebis (dimethylalkylammonium) salts Rossum and Ariëns[50] found

that the introduction of a single alkyl group alters the type of action. As the series is ascended from the methyl to the decyl derivative, the high intrinsic activity of decamethonium disappears, and the affinity for non-competitive receptors increases. It is interesting to note that the higher members of the series have a high affinity for both acetylcholine and non-competitive receptors and are described as drugs with a dualism in antagonism. This type of action is quite distinct from the "dual" block described by Zaimis,[43] since drugs exhibiting a "dual" block have a moderate intrinsic activity and are presumed to react with only acetylcholine receptors. According to Rossum and Ariëns the change from a depolarising to a competitive action, with the introduction of homologous alkyl groups, is due to a reduction of the effectiveness with which the positively charged onium group can interfere with the electrical field of the receptor. This effect is mediated, largely, through a decrease in the charge density of the onium ion, but also by steric factors, such as the masking of the onium ion by electro-repulsive alkyl groups. With larger alkyl groups, such as n-pentyl and n-hexyl, the increase in surface activity and lipoid solubility induce an affinity for non-competitive receptors. The ratio of the affinities for acetylcholine and non-competitive receptors determines whether competitive or non-competitive features will be exhibited first.

(ii) Aromatic Substituents.

The influence of a single aromatic substituent generally parallels, but is more pronounced than that produced by the introduction of larger alkyl groups in both the methonium and ethonium series. The compounds produced by the substitution of one methyl group in decamethonium by a benzyl or p-nitrobenzyl radical have a mixed action. Thus, in the chicken and mouse the decamethylenebis-benzyl and -p-nitrobenzyl-dimethylammonium derivatives produce a paralysis without a contracture, but the benzyl derivative does not appear to be antagonised by neostigmine, and the p-nitrobenzyl derivative is antagonised by all dose levels of neostigmine in the mouse only.(65) In the polymethylenebis(p-nitrobenzyldimethylammonium) series maximum activity, comparable with (+)-tubocurarine when tested on the cat, was found in the nonamethylene member.(68) The hexamethylene member was only about one-quarter as active, but hexamethylenebis(p-nitrobenzyldiethyl-ammonium) was equi-active with (+)-tubocurarine. Cavallito and his colleagues(69) have also found that with the introduction of larger, aromatic groups, such as benzhydryl, fluorene, carbazole, or phenothiazine derivatives, maximum activity resided in the hexamethylenebis-(9-fluorenyldimethyl-ammonium) and -(9-fluorenyldiethylammonium) compounds (XII A and B).



A (XII) B

These were competitive blocking agents and about equi-active when tested on the mouse inclined screen and dog tibialis nerve-gastrocnemius muscle preparations. These compounds also have a very high anti-cholinesterase activity which has led to the use of (XIIA, "hexafluorenium", "mylaxen") as a potentiator for succinylcholine and other short acting muscle relaxants.(70)

The effects of aromatic substitution invite some speculation about the receptor and the mode of drug attachment to it. Assuming that the receptor site will have a three dimensional structure, then a drug containing an aromatic substituent could bind as shown in Figure 1.

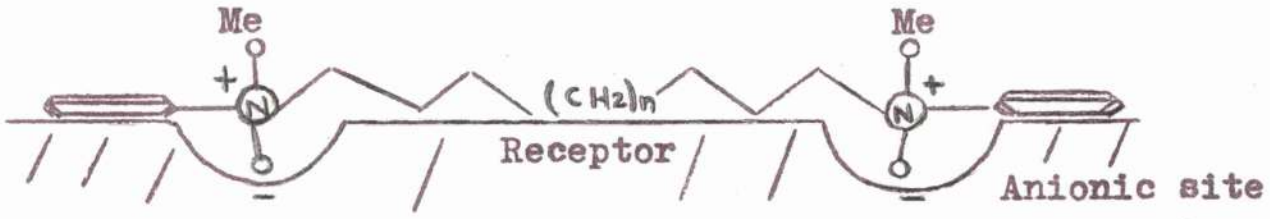


Fig.1

The chain and the flat aromatic ring would then be available for van der Waals' bonding, and Cavallito has attributed the high activity of the 9-fluorenyl derivatives

to this effect. The increased activity of the p-nitrobenzyl over the benzyl derivatives mentioned previously may be due to the -I effect of the electron attracting nitro-group which would increase the charge density on the onium ion. The other possible arrangement is that in Figure 2, in which

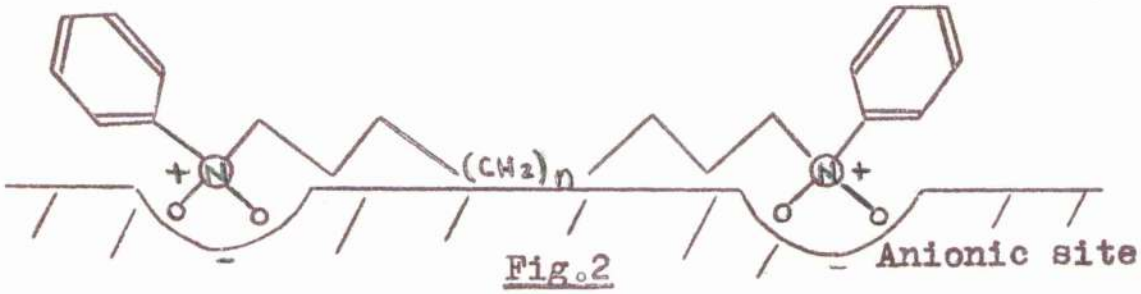


Fig.2

the drug molecule would be presenting the same cationic head to the receptor as in decamethonium and would therefore be expected to exhibit stimulating properties. If, on the other hand, the stimulating properties of decamethonium are due to displaced acetylcholine, the benzene ring could have a shielding effect which might prevent the displacement of the drug molecule by acetylcholine. Such an "umbrella" effect has been attributed to the tropic acid residue of atropine and related parasympathetic blocking agents.(25a) But Rossum and Ariëns(50) have found that decamethylenebis (isopropyldimethylammonium) has a low intrinsic activity while the n-propyl analogue is devoid of intrinsic activity. Since the isopropyl radical should provide better shielding properties than the n-propyl radical the absence of stimulating properties because of this effect could be

discounted. Hence the formation of the drug receptor complex in Figure 2 would seem improbable. The importance of van der Waals' bonding and the position of electronegative substituents in the aromatic ring are clear from Figure 1. The close attachment of the aromatic substituents to the receptor surface could possibly prevent any configurational changes necessary for depolarisation of the membrane, as proposed by Nachmansohn.[33a] The compounds containing larger alkyl groups probably combine with the receptor in a similar fashion.

(iii) Heterocyclic Compounds.

Many workers have incorporated the nitrogen atom of the polymethylene compounds into an aromatic or reduced cyclic system. The aromatic compounds afford greater ease of approach to the receptor but have a much lower charge density on the onium ion than their reduced analogues, and, because of this are generally much less active. This generalisation and also the importance of substituents in the ring are exemplified by Taylor's findings, shown in Table 1.[71] Since close approach to, and possibly envelopment by, the receptor appears to be a prerequisite for depolarising properties, the replacement of two methyl radicals in decamethonium by a small, compact heterocyclic ring, as for example, pyridine, piperidine, pyrrolidine, or morpholine would not be expected to alter the type of

$R^{+}\cdot(CH_2)_{10}\cdot R^{+}$ 2x'		
A. Nature of R^{+}	Rabbit	Mouse
Quinolinium	4.5	0.9
1,2,3,4-Tetrahydro-1-methylquinolinium	0.75	0.9
trans-Decahydro-1-methylquinolinium	0.1	0.75
Isoquinolinium (bromide)	ca.4.0	3.6
1,2,3,4-Tetrahydro-2-methylisoquinolinium	1.5	1.9
cis-Decahydro-2-methylisoquinolinium	<0.5	0.7
Pyridinium (bromide)	-	1.9
1-Methylpiperidinium	-	2.0
1-Methylpyrrolidinium	-	1.25-1.5
B.		
6,7-Dimethoxyquinolinium	0.15	0.4
6,7-Dimethoxyisoquinolinium	>1.0	1.8
1,2,3,4-Tetrahydro-6-methoxy-1-methylquinolinium	0.2	1.0
1,2,3,4-Tetrahydro-6,7-dimethoxy-1-methylquinolinium	0.08	0.65
1,2,3,4-Tetrahydro-6-methoxy-2-methylisoquinolinium	0.2	0.9
1,2,3,4-Tetrahydro-6,7-dimethoxy-2-methylisoquinolinium	0.05	0.35
1,2,3,4-Tetrahydro-6,7,8-trimethoxy-2-methylisoquinolinium	0.02	0.3

Table 1. Minimum effective dose and effective $dose_{50}$ in mg./Kg. body weight of decamethylenebisonium salts in the rabbit and mouse, respectively.

action. A piperidine ring would probably offer less steric hindrance to a receptor cavity as depicted in Figures 1 and 2 than two ethyl radicals, and there would be far less shielding of the onium charge than in the unusual "swastika-like" conformation suggested for the alkyltriethylammonium "head" by Carey, Edwards, Lewis, and Stenlake.[37] This is based on the conformation of the tetraethylammonium ion established by the X-ray studies of Wait and Powell[114] (Figure 3).

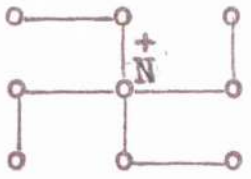
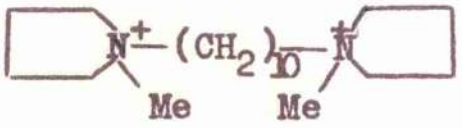


Fig.3.

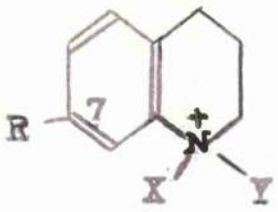
Thus Thesleff and Unna[65] found that pyridine and pyrrolidine substitution did not alter the depolarising properties of decamethonium and, in an extensive series of polymethylenebis-morpholinium, -piperidinium and -pyrrolidinium salts, Wien and Mason[72] found that the most active compound, the decamethylenebis(1-methylpyrrolidinium)dihalide (XIII), had predominantly decamethonium-like properties on the cat gastrocnemius muscle and



(XIII)

rabbit diaphragm preparations. Again, this result could have been predicted on the basis of the smaller pyrrolidinium "head" providing easier access to the receptor than the six-membered rings. Substitution in the 2 and 5 position of the pyrrolidine ring increases the size of the onium "head" and decreases activity.(73)

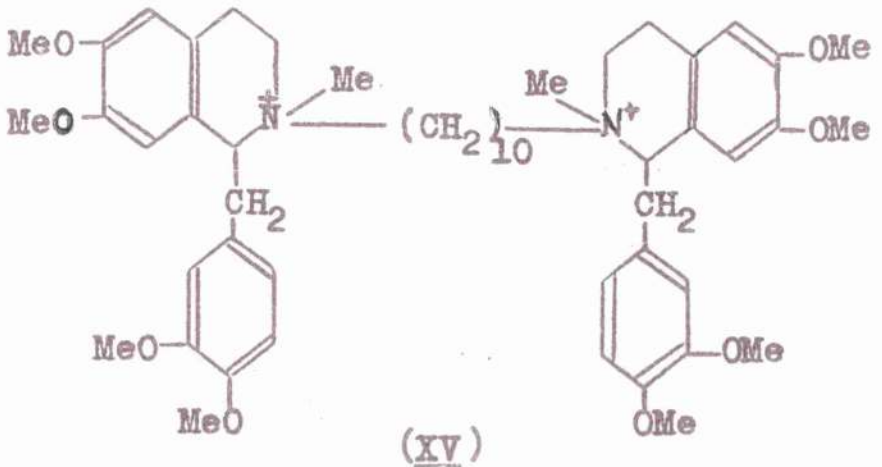
In contrast to the above findings all the compounds in Table 1, section B, in which the onium nitrogen forms part of a large, substituted heterocyclic unit, have a (+)-tubocurarine-like action on the chick and rabbit.(74,71) The optimal chain lengths for the last two compounds in section B are 10 or 11, and 10, respectively. The reason for the marked increase in activity associated with the methoxyl groups is not clear. In quinoline and isoquinoline derivatives the electron-attracting (-I) effect on the π electrons of the ring may increase the charge density of the onium ion, but it is very difficult to predict the effect on the C - N sigma bond in their tetrahydro-analogues. Taylor (D.B.)(40) has suggested an experimental approach to this problem by observing the biological effect of nitro- and chloro-substitution in position 7 in (XIV).



(XIV)

Other effects which might contribute to activity are a reduction in the polarity of the molecule, which reason Cavallito[28] has proposed for the enhanced activity of the O O-dimethyl ether of (+)-tubocurarine, or involvement with receptor structures in some undetermined way.

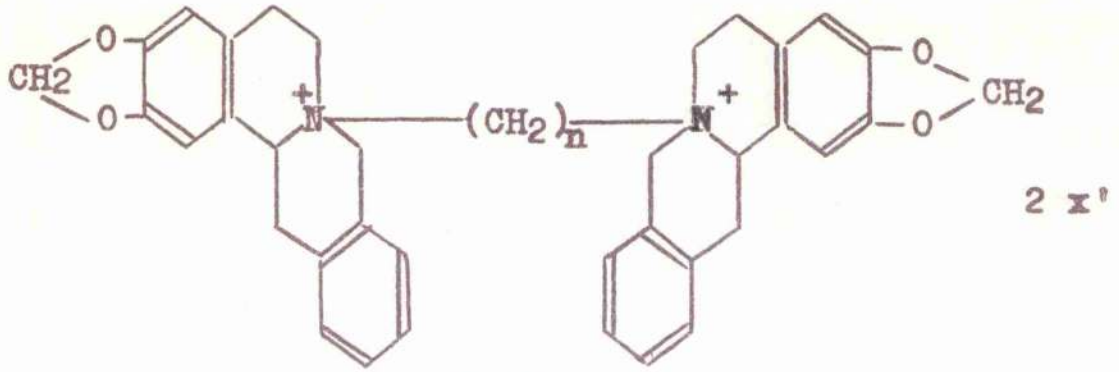
Further work on methoxyl substituted compounds by Collier and Taylor[75] led to the synthesis of "Laudexium" (XV) which is approximately four times as active as (+)-tubocurarine in the rabbit, and has an almost pure competitive action on the chick.[76] The laudanosine moiety closely



(XV)

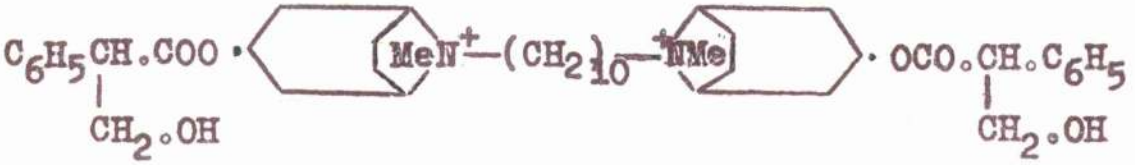
resembles half of the molecule of (+)-tubocurarine. Other workers[77] have studied polymethylenebis-β-carboline, -α-carboline, -yohimbine and -tetrahydroberberine derivatives whose ring structures also resemble those of the curare

alkaloids. The most active of this group of compounds were the hexa- and deca- methylenebis(tetrahydroberberine) derivatives (XVI $\underline{n}$ = 6, 10) which were competitive blocking agents with a moderate activity.



(XVI)

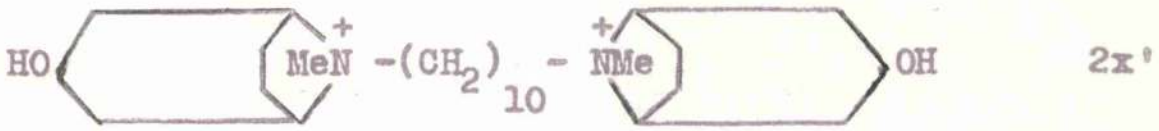
The tropane alkaloids also provide some active competitive agents. Kimura and Unna[78] have found the decamethylenebisatropinium salt (XVII) to be more active than its pentamethylene analogue, while Eckfeld[79] has also



(XVII)

found for hexamethylene- and octamethylene-bis-atropinium and-scopolaminium salts that the longer chain compounds are the more active. However, these compounds retain many of the properties of their parent bases. Haining and Johnston[80] have examined the influence of chain length and of the ester group in a series of bistropëines. They found that

esterification was vital for high activity in the N N'-decamethylenebis(tropinium)dihalide (XVIII), and that



(XVIII)

the nature of the esterifying acid could also influence activity to some extent. Thus, mandelic acid was more effective than tropic, benzoic, or phenylacetic acids, in this capacity. These workers suggested that the main role of the ester group was to provide active centres which could be attracted by the receptor surface. There was little difference in activity when the linking chain contained from seven to twelve methylene groups, and the compounds were generally more potent than (+)-tubocurarine on the cat and rabbit.

B. Chain-substituted Bisonium Salts.

(i) Esters

An ester group is susceptible to hydrolysis by the cholinesterases and can decrease the duration of action of a drug. Rossum and Ariëns have suggested[50] that the high intrinsic activity of succinylcholine may be due to the electronegative ester group increasing the positive charge on the nitrogen, but this seems improbable since any polarization would be expected to be in the direction of the quaternary nitrogen in Figure 4.

$$-(CH_2)_2-\overset{O}{\overset{\|}{C}}-O-(CH_2)_2\cdot\overset{+}{N}Me_3$$

Fig.4.

Succinylcholine (XIX) is a powerful depolarising agent of short duration which is used clinically. It was synthesised

$$Me_3\overset{+}{N}.CH_2CH_2.OCO.(CH_2)_2.COO.CH_2CH_2.\overset{+}{N}Me_3 \qquad 2\ I'$$

(XIX)

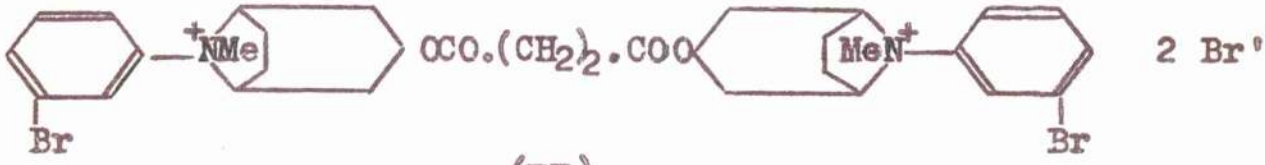
by Bovet's school in 1949.[81] As the dicholine ester of succinic acid it can be regarded as virtually a double molecule of acetylcholine, and has a chain length approximately equal to the ten methylene units of decamethonium. The overall effect of alkyl substitution is similar to that found in decamethonium, but the pattern is different. Whereas, the introduction of one ethyl radical into decamethonium produced an immediate large fall in activity, a pronounced decline in activity is seen in succinylcholine only when two ethyl radicals have been introduced. The effect of progressive methyl substitution on the rabbit head drop dose (H.D.D.) is shown in Table 2.[82]

$R.(CH_2)_2.OCO.(CH_2)_2.COO.(CH_2)_2.R$				
R	$Me_3\overset{+}{N}$	$Me_2\overset{+}{N}Et.$	$MeN\overset{+}{E}t_2$	$Et_3\overset{+}{N}$
H.D.D. Mg/Kg.	0.2	0.8	20	12

TABLE 2

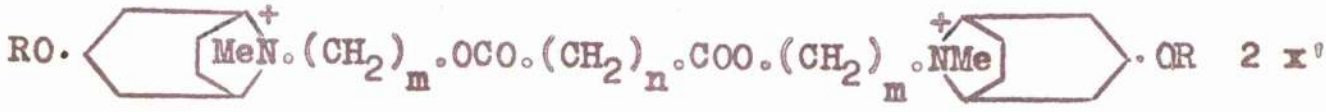
Similar results were obtained on the rat phrenic nerve diaphragm preparation.[83] Adipoylcholine, the dicholine ester of adipic acid, which is about one half as active as succinylcholine on the rabbit head drop test, is similarly affected by analogous alkyl substitution.[82] The bis-triethylammonium derivative produces a (+)-tubocurarine-like paralysis of pigeons, and fails to produce a contracture of the isolated frog rectus abdominis muscle.[83a]

Attempts to produce reversible blocking agents with a short duration of action have led to the synthesis of compounds with two ester groups, as in succinylcholine, and large heterocyclic terminal groups. Bistropine derivatives, linked through the 3-hydroxyl position by esterification with various dicarboxylic acids, such as succinyldi(m-bromobenzyltropinium)di-iodide (XX) provide competitive compounds of



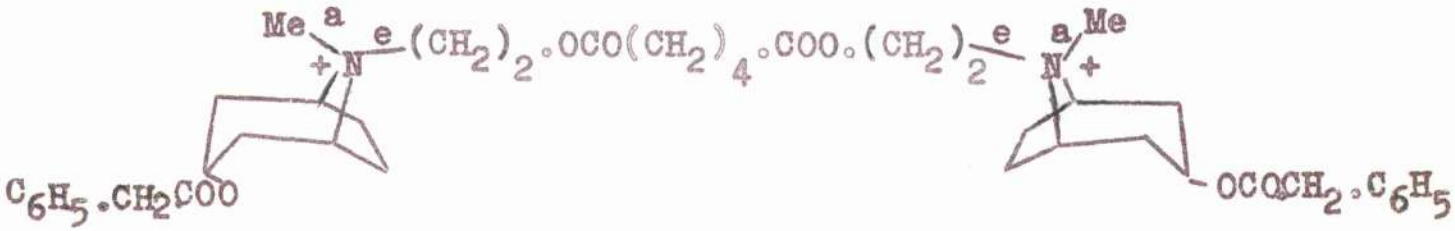
(XX)

short duration and high activity.[84] Similar results were obtained by Haining and his colleagues[85] in a number of tropeines (XXI, m = 2,3; n = 0-6, R = ester group).

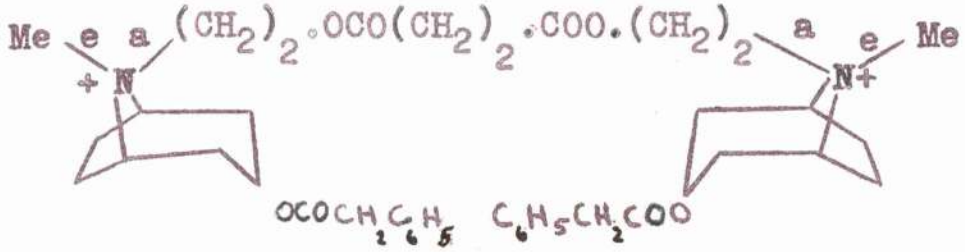


(XXI)

As in the polymethylene series, potency was dependent on the chain length and, to some extent, on the nature of the aromatic esterifying acid. Epimerisation in (XXI, m = 2, n = 4, R = phenylacetyl = XXII) and in other members of the series, whereby the relative positions of the methyl radical and the chain were reversed, decreased activity but only slightly on the cat gastrocnemius muscle preparation. If the ester groups are involved in bonding with receptor structures, as suggested previously by these workers, their altered orientation, as shown below, would have been expected to reduce activity more significantly.



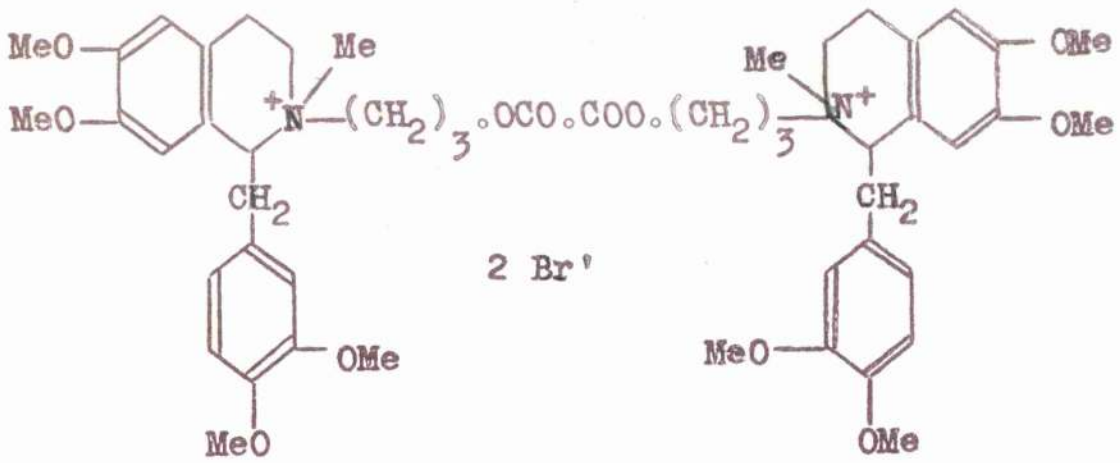
(XXII)



(Epimer of XXII)

The conformation of the tropane ring is based on that depicted by Fodor.[86] The piperidinium analogue of (XXII) has a depolarising action on the isolated frog rectus abdominus muscle and on the chicken, but replacement of the N-methyl by an N-benzyl radical restores competitive action.

Compounds related to "Laudexium" and to succinylcholine have been reported by Gladych.[87] Only the diester (XXIII) had a briefer action than succinylcholine, but it had only about one-thirtieth of its potency.[88]



(XXIII)

(ii) Ethers.

Ethers are generally less active than the polymethylene compounds from which they are derived, and the overall chain length does not appear to be as important. The much lower activity of morpholinium compounds, as compared to their piperidinium analogues, is generally regarded as being due to the polarisability of the lone pair of electrons on the

ether oxygen atom delocalising the positive charge on the onium nitrogen. Hydration has also been mentioned as a possible mechanism.[35] In the following examples no mention was made of the type of action shown, but the fact that an increase in the size of the onium head generally increases the activity would suggest that they are competitive agents. Table 3 shows the effect on the paralysing doses (mg./Kg.) for the rat phrenic nerve diaphragm(R.N.D.), rat gastrocnemius muscle(R.G.M.), and rabbit head drop(H.D.) preparations, caused by onium substitution in 1,10-decamethylenebis(trimethylammoniumethoxy)di-iodide (XXIV).[89]

$$Me_3\overset{+}{N}.(CH_2)_2.O.(CH_2)_{10}.O.(CH_2)_2.\overset{+}{N}Me_3 \qquad 2\ I^-$$

(XXIV)

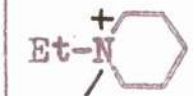
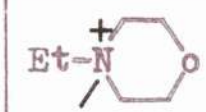
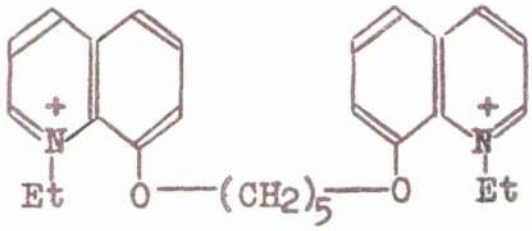
$-\overset{+}{N}R_3$	R.N.D.	R.G.M.	H.D.
$-\overset{+}{N}Me_3$	2.3	1.9	0.8
$-\overset{+}{N}Et_3$	1.2	1.1	0.12
	0.6	0.96	0.09
	4.6	3.5	0.2

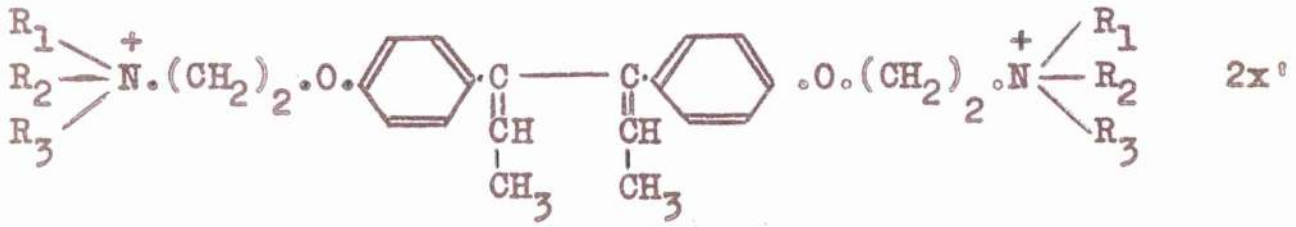
TABLE 3

A similar order of activity was found on substitution in 1,2-ethylenebis(trimethylammoniumethoxy)di-iodide,[90] and Pradhan[91] has found that the activity is further increased by the introduction of 1-butyl- and 1-benzyl-piperidino radicals. However, slightly different results are obtained when ring structures are included in the chain. Thus in 1,5-pentamethylenebis(1'-ethyl-8'-oxyquinolinium)di-iodide (III), replacement of the N-ethyl radicals



III

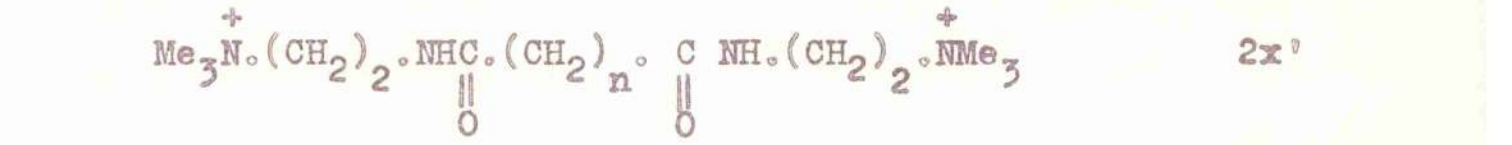
by n-propyl or n-butyl decreases the activity on the rabbit head drop test,[82b] and in the dienoestrol derivatives (XXV) the order of activity for onium substituents by the same test is triethyl = diethylmethyl > 1-methylpiperidino.[92]



(XXV)

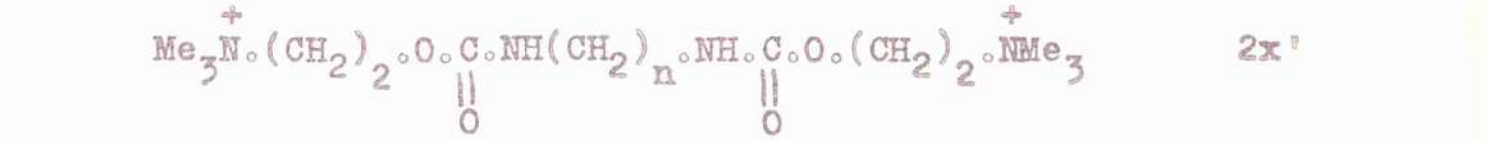
(iii) Amides.

The amides, of general structure (XXVI), are weak neuromuscular blocking agents.[93] Rossum and Ariëns[50]



(XXVI)

have found for this series that a reduction of the chain length, or onium substitution decreases the intrinsic activity, producing compounds with a competitive action. However, the carbamylcholine derivatives (XXVI A) are depolarising



(XXVI A)

agents with a high intrinsic activity.[50] The amide nitrogen of secondary amides may bear a positive charge due to a resonance effect (Figure 4 a).

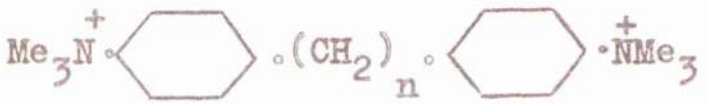
Fig. 4a

Consequently the proximity of this positive charge to the onium nitrogen would decrease its ability to interact with the receptor and could account for the low intrinsic activity of amides (XXVI) as compared to esters or carbamylcholine derivatives.

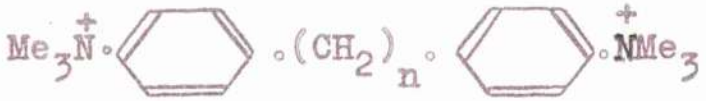
(iv)(a). Ring-containing Chains.

Apart from reducing the possible conformations of a flexible hydrocarbon chain ring structures can influence

the closeness of approach of the onium "head" of a molecule to the receptor. Thesleff and Unna[65] have shown that, independent of chain length within a limited series, the replacement of methylene units by two cyclohexane groups (XXVII, n = 0-2) produces compounds with a (+)-tubocurarine-like action, but their replacement with two phenyl groups (XXVIII, n = 1-3) preserves the decamethonium-like action. The flat benzene ring will allow a closer



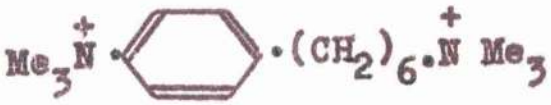
(XXVII)



(XXVIII)

approach to the receptor than the non-planar cyclohexane ring. The replacement of one methyl group by an ethyl or benzyl radical in the diphenyl compound (XXVIII, n = 2) abolishes its contracture producing properties. The paralysis produced by the ethyl derivative is readily antagonised by tensilon or eserine in the chicken, and by neostigmine in the mouse, while the benzyl derivative is also antagonised by neostigmine in mice. These findings are confirmed by the results of other workers. Wien and Mason[94] found phenylhexane p-ω-bis (trimethylammonium)di-iodide (XXIX) to be a depolarising agent

equi-active with (+)-tubocurarine on the cat gastrocnemius muscle preparation, but the bistriethylammonium analogue, although as active, was a competitive agent.



2 I'

(XXIX)

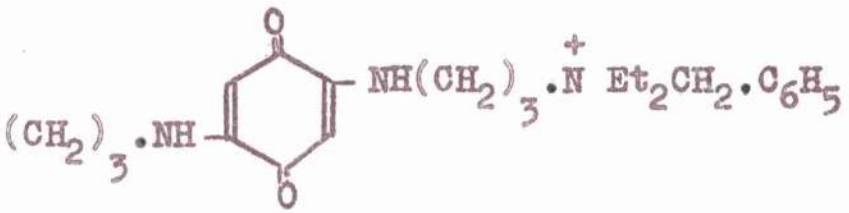
The 1,4-piperazine derivatives (XXX)[95] have little activity, but the three most

2x'

(XXX)

active compounds, $R_3\overset{+}{N}$ = 1-methylpiperidinium, $R_3\overset{+}{N}$ = 1-ethyl-piperidinium, $R_3\overset{+}{N}$ = triethylammonium produce a competitive block on the rabbit head drop, frog rectus abdominus and rat phrenic nerve diaphragm preparations.

The diamino-benzoquinone derivative, "Mytolon", (XXXI), has a predominantly competitive action in most species, including man.[96] It has been suggested that the high activity of



2 Cl'

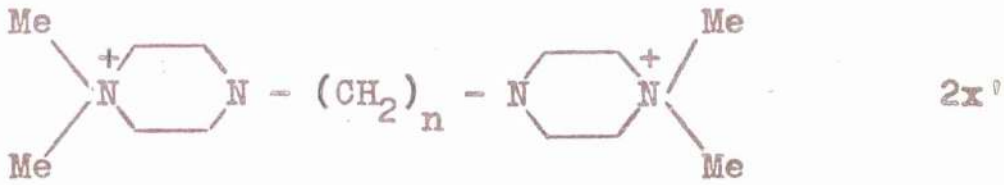
(XXXI)

aminobenzoquinone derivatives is due to chelation of the quinone structure with metalloporphyrins associated with the

receptor.[96a] Onium substitution in "Mytolon" has been investigated by Hoppe and his colleagues[97] on nerve muscle preparations of the cat and dog, and on the mouse. Replacement of the benzyl groups by ethyl radicals resulted in a two to five-fold decrease in potency in the cat and dog. Successive replacement of the ethyl by methyl radicals also reduced activity; the bistrimethylammonium compound being the least potent member of the series except in the cat, on which it had a decamethonium-like action.

(iv)(b) Rings Common to the Onium Head and Chain.

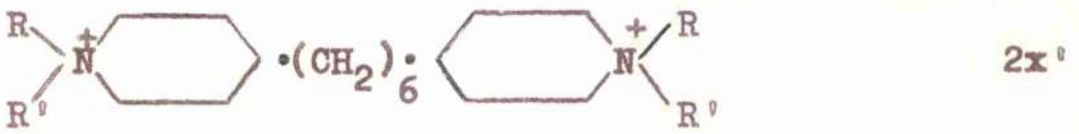
The structure-action relationships of this class of compounds resemble those of the heterocyclic polymethylene compounds. The bispiperazine derivatives (XXXII, n = 6, 10)[98] have a depolarising action on the chicken gastrocnemius muscle preparation, but in both



(XXXII)

cases the bis(diethylpiperazinium) analogues produce a competitive block associated with a reduction in activity; while the decamethylenebis(methylbenzylpiperazinium) derivative reverses the type of block and also enhances the activity on the rabbit head drop test. In the

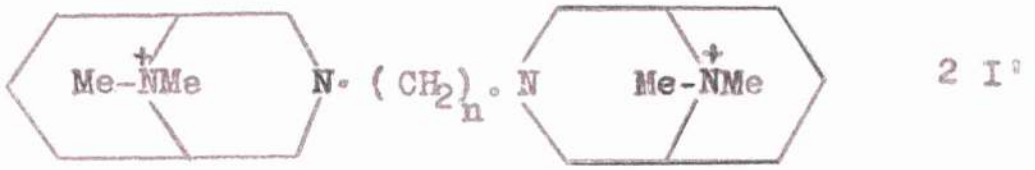
bispiperidine derivatives examined by Randall[99] (XXXIII), when R = R' = methyl, the compounds



(XXXIII)

have an action and potency similar to decamethonium, but the derivatives in which R' = lower alkyl, and R = aralkyl, such as p-nitro-phenyl, or -benzyl, have a tubocurarine-like action.

The polymethylenebis [9-methyl-3, 9-diazabicyclo-(3,3,1) nonanes] (XXXIV, n = 4-6),[115] whose ring structure resembles tropane, provide some active compounds. The most



(XXXIV)

active member (XXXIV, n = 4) has an inter-onium distance approximately equal to ten carbon atoms. It is more potent than decamethonium and has a duration of action on the cat intermediate between that of succinylcholine and decamethonium. It is unusual in that it has a depolarising action on the cat but a competitive action on the frog rectus abdominus muscle.

POLYONIUM SALTS .

By comparison with the bisquaternary salts polyonium compounds have been neglected both in the numbers prepared and in the study of their structure-action relationships. Gallamine triethiodide, the first member of this group, is widely used clinically. It was synthesised by Bovet and his colleagues in 1947 during the examination of the neuromuscular blocking properties of mono- and poly-phenolic ethers of certain aminoalcohols. The increase in activity and duration of action with the introduction of the third onium group in compounds of this series is shown in Table 4.

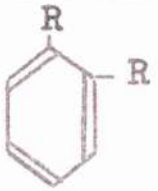
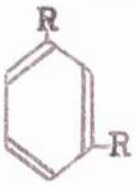
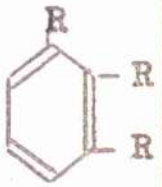
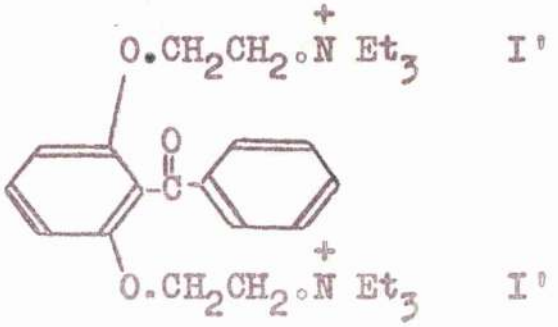
R = O $CH_2 . CH_2 . \overset{+}{N}\ Et_3\ I^-$.					
Compound					
Rabbit Head drop dose(mg/kg)	20	1.5	1	3.5	0.5
Duration of action	20 min.	1 hr.	1 hr.	1 hr.	1hr.45 mins.

TABLE 4

However, Unna[100] has pointed out that the middle onium group is not essential for high activity and that its prime function in gallamine is probably to modify the freedom of rotation of the two side chains, maintaining them at an optimal distance, without itself being actually involved in

bonding. As evidence of this, he has described the high activity of 2,2,6-bis(diethylaminoethoxy)benzophenone diethiodide (XXXV) which is about one and a half times more potent than gallamine and has a similar type of action.



(XXXV)

Gallamine is a "pure" competitive blocking agent with a potency about one quarter that of (+)-tubocurarine on the cat, although the ratio of their activities varies in different species.[101] Replacement of the ethyl radicals with methyl decreases the activity, but this is not significant until at least two of the ethyl groups on each onium ion are replaced, and the type of action remains almost unchanged. The tristrimethyl ammonium analogue produces some depolarisation of the frog sartorius muscle but this is not comparable, in either intensity or duration, to that of decamethonium.[101]

In a series of tris- and tetra-dialkylaminomethylbenzene ethiodides, Table 5, Funke and co-workers[102] have shown that the relative positions rather than the number of quaternary groups is important.

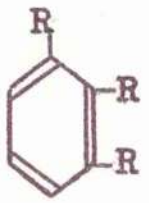
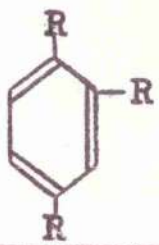
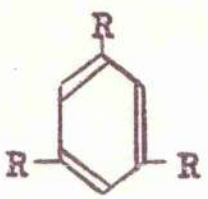
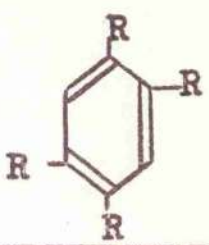
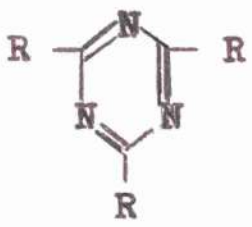
R = $CH_2\overset{+}{N}Et_3$				
Compound				
Rabbit Head-Drop Dose (mg/kg)	2.5	2.5	5	15

TABLE 5

This is borne out by the low activities of the trisquaternary ammonium salts in the sym-triazine series (XXXVI) which are less active than the corresponding bisquaternary salts.(103)



R = $NH(CH_2)_n NR'_2 R''$

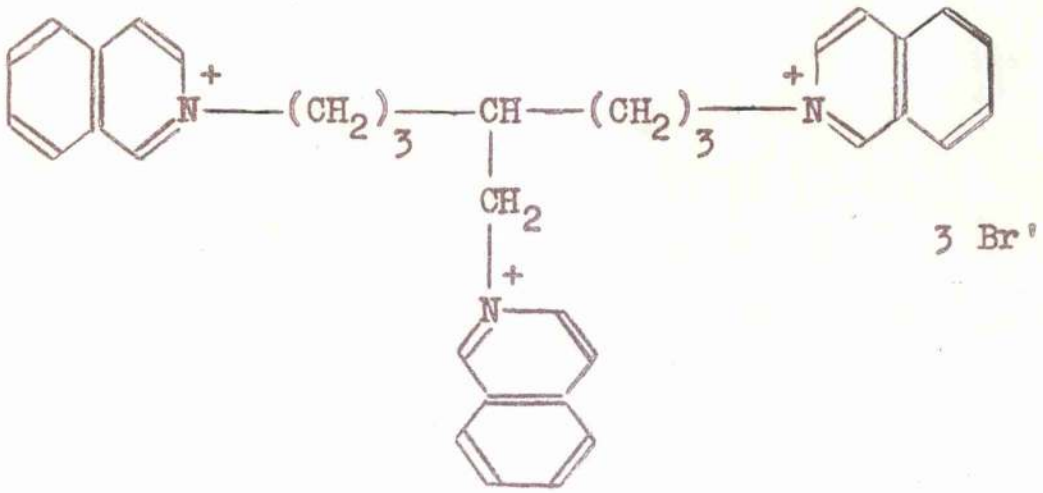
n = 2,3

R' = lower alkyl

R'' = R' or benzyl

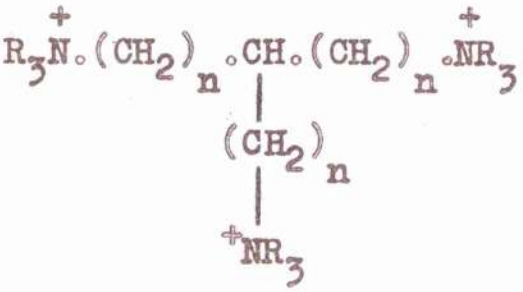
(XXXVI)

A very interesting compound is the trisisoquinoline derivative, Curaroid I (XXXVII),(104) which is



(XXXVII)

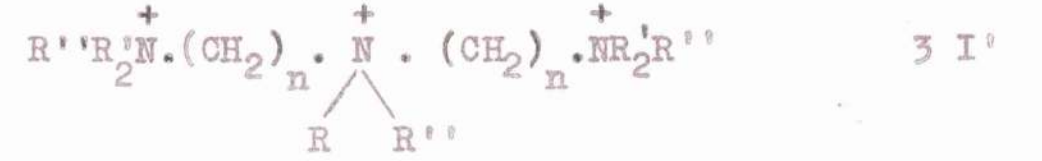
equi-active with (+)-tubocurarine on the rabbit head drop test, and about twice as active on the dog gastrocnemius muscle. Its very high activity is in direct contrast to the feeble properties of the penta- and deca-methylenebis (quinolinium) compounds tested by Barlow and Ing[64] and Taylor,[71] and cannot readily be explained by the influence of the mid-onium group on the orientation of the other two groups. Cavallito has stated that curare-like properties appear to be more significant among polyquaternary salts where the onium groups are joined in a more linear rather than in a multiple appendage arrangement.[28] The trisonium compounds of Kensler and co-workers (XXXVIII), n = 2-4, R = Me or Et) have a similar structure to Curaroid I,



(XXXVIII)

but the most active compound (XXXVIII), $\underline{n} = 4$, $R_3 = Et_3$) was only about two-fifths as active as gallamine.[105]

The short chain linear trisquaternary ammonium salts (XXXIX), $\underline{n} = 2,3$; R,R',R'' = lower alkyl) are ganglion blocking agents.[106,107]



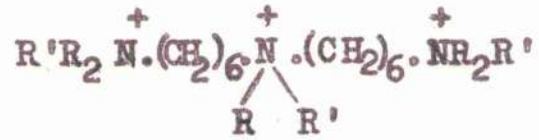
3 I'

(XXXIX)

but their longer chain homologues exhibit considerable neuromuscular blocking activity.[108,109,37] Stenlake and his colleagues have studied linear N S N-trisonium,[108,110,37] N N N N-tetraonium, N N S N N-pentaonium and N N N N N N-hexaonium salts[109,111,112] and also linear tris- and tetra-onium ethers.[113] These compounds were tested for their neuromuscular blocking activity on the rabbit, mouse, chick and isolated frog rectus abdominus muscle, and, for their duration of action on the cat and chick. The main features of these compounds are that the inter-onium distance, rather than the overall chain length, determines the type of block, while the number of onium groups in the molecule influences potency, reversibility and duration of action. Thus tris-tetra-, penta-, and hexa-onium compounds, in which the onium groups are separated by five or six methylene units, are predominantly competitive agents. Compounds in which the onium groups are separated by eight methylene units have

mixed effects, while those having ten methylene units between the onium centres are predominantly depolarising agents, (Table 6).

The dihexazonium (XL) and dihexasulphonium (XLI) compounds are compared in Table 7. It will be seen that



(XL)

(XLI)

the stepwise replacement of ethyl by n-propyl radicals produces a steady reduction in potency, but a single n-butyl group enhances activity. From their findings in this series these workers were led to suggest that the receptor surface presented a repeating pattern of anionic sites, 9 to 10 A° apart, each being associated with possibly two or three, non-ionic satellite receptors complementary in size and shape to the ethyl substituents.

Table 6. Comparison of chain length of poly-onium compounds with type of neuromuscular block exhibited

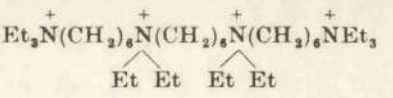
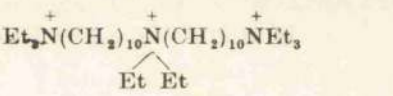
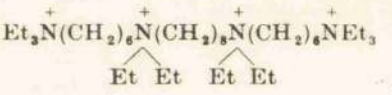
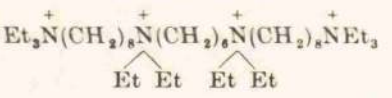
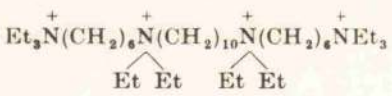
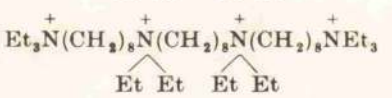
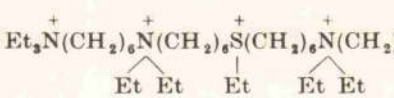
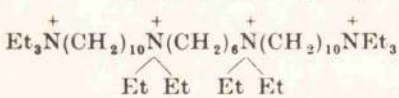
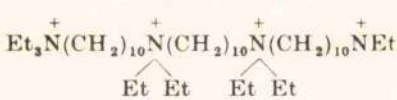
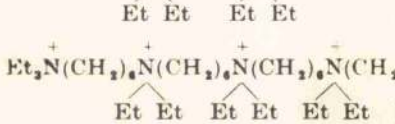
Structure	Type of block	Number of atoms separating terminal onium groups
	TC-like	20
	C 10-like	21
	TC-like	22
	TC-like	24
	TC-like	24
	Transitional	26
	TC-like	27
	C 10-like	28
	C 10-like	32
	TC-like	34

TABLE 7.

THE INFLUENCE OF CHANGES IN ALKYL GROUP SIZE UPON NEUROMUSCULAR BLOCKING ACTIVITY IN THE CAT, RABBIT, MOUSE, AND FROG

COMPOUND			Cat	Rabbit	Mouse	Frog
$\overset{+}{N}(CH_3)_3\ \overset{+}{S}(CH_2)_6\ \overset{+}{N}$			TC = 100	TC = 100	TC = 100	TC = 100
Me_3	Me	Me_3	20	14	5	—
Me_2Et	Et	Me_2Et	26	27	24	14
Me_2Bu	Bu	Me_2Bu	44	33	24	10
$MeEt_2$	Me	$MeEt_2$	87	46	42	16
Et_3	Et	Et_3	95	30	25	25
Et_2Pr	Pr	Et_2Pr	46	51	54	16
$\overset{+}{N}(CH_3)_3\ \overset{+}{N}(CH_2)_6\ \overset{+}{N}$						
Et_2Me	EtMe	Et_2Me	50	52	16	29
Et_2Pr	EtPr	Et_2Pr	88	69	48	16
Et_2Bu	EtBu	Et_2Bu	120	155	63	47
Et_3	EtEt	Et_3	100	21	17	50
$MePr_2$	MePr	$MePr_2$	15	18	11	5
$EtPr_2$	EtPr	$EtPr_2$	31	46	17	15
Pr_3	PrPr	Pr_3	20	21	3	13

Pr = *n*-propyl. Bu = *n*-butyl

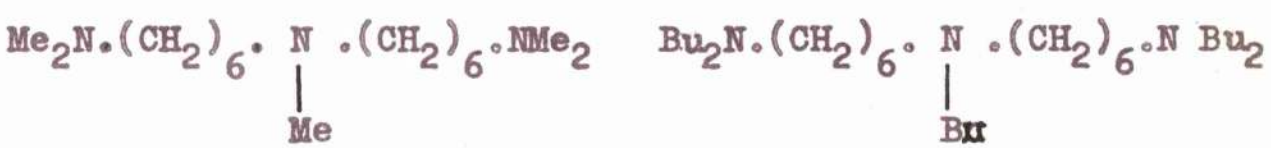
PART I

D I S C U S S I O N

Object of syntheses undertaken.

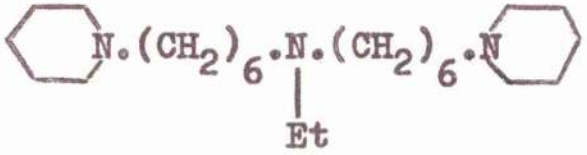
Stenlake and Lewis and their colleagues, as mentioned previously,[109] have shown the overriding influence of chain length in determining the type of action displayed by the polyonium neuromuscular blocking agents. As with the methonium compounds the nature of the onium substituents has been found to have a marked effect on potency.[37,109] However, relatively few substituents have been studied, and these mainly in the N N N and N S N dihexazonium and dihexasulphonium compounds. The findings so far confirm that the larger alkyl groups favour (+)-tubocurarine - like activity, and the increase in potency on the successive replacement of methyl by ethyl groups (Table 7) in both dihexazonium and dihexasulphonium parallels the influence of similar replacement in gallamine.[101]

The object of the experimental work described in the first part of this thesis was to extend the studies on onium substitution in the N N N - dihexazonium compounds. To the quaternary salts of the ethyl and n-propyl bis(6-dialkyl-aminohexyl) alkylamines prepared by Carey, Edwards, Lewis and Stenlake[37] are now added those from the methyl (XLII) and n-butyl (XLIII) triamines,

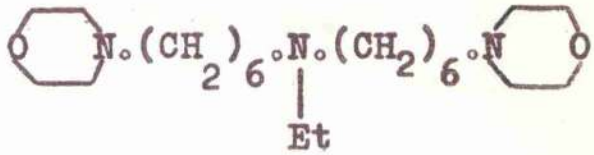


(XLII) (XLIII)

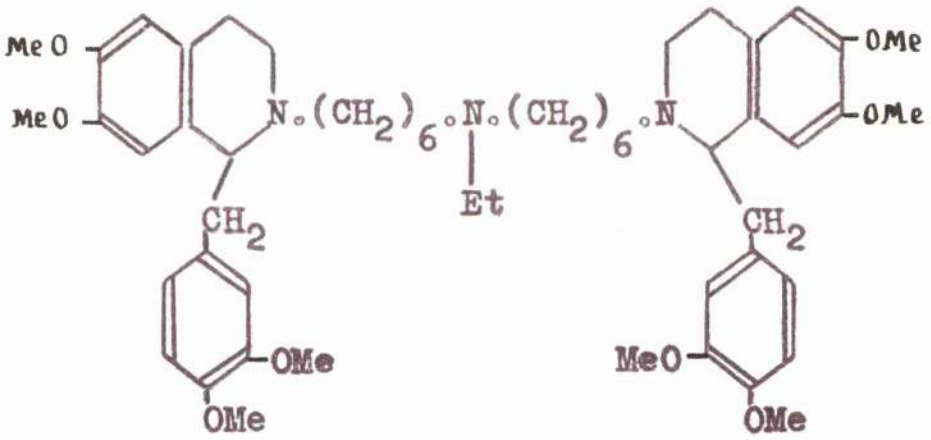
and from bis(6-piperidinohexyl) -, bis(6-morpholinohexyl) - and bis(6,2'-tetrahydropapaverinylhexyl)-ethylamine (XLIV to XLVI resp.)



(XLIV)



(XLV)



(XLVI)

Although Corrodi and Hardegger[116] have resolved tetrahydropapaverine into its d and l forms in the course of their successful work on the absolute configuration of laudanosine, only the racemate was employed in the synthesis of the triamine (XLVI).

DISCUSSION OF EXPERIMENTAL WORK.

The short chain linear compounds of Delaby, Damiens and Marquist [107] were prepared by the condensation of the 2-dialkylaminoethyl chloride with a primary amine to give either di- or tri- amines according to the equations,

A. $R_2'.(CH_2)_2.Cl + 2\ R.NH_2 \longrightarrow R_2'N.(CH_2)_2.NHR + RNH_2.HCl$

B. $2\ R_2'N.(CH_2)_2.Cl + RNH_2 \longrightarrow R_2'N.(CH_2)_2.\underset{\displaystyle R}{N}.(CH_2)_2.NR_2'.2HCl$

while the route to the hexamethylene compounds (Ll = XL, R = Et_2) originally chosen by Edwards, Lewis, Stenlake and Zoba[108] utilised 6-hydroxyhexyldiethylamine (XLVII) as the key intermediate.

$$Et_2N.(CH_2)_6.OH \xrightarrow{HBr-H_2SO_4} Et_2N.(CH_2)_6.Br \xrightarrow{EtNH_2} Et_2N.(CH_2)_6NHEt$$

(XLVII) (XLVIII) (XLIX)

$Et_2N.(CH_2)_6.Br$

$$R'Et_2\overset{+}{N}.(CH_2)_6.\underset{\displaystyle R'\ \ Et}{\overset{+}{N}}.(CH_2)_6.\overset{+}{N}Et_2R' \xleftarrow{R'I} [Et_2N.(CH_2)_6]_2N\,Et$$

3 I'

(LI) (L)

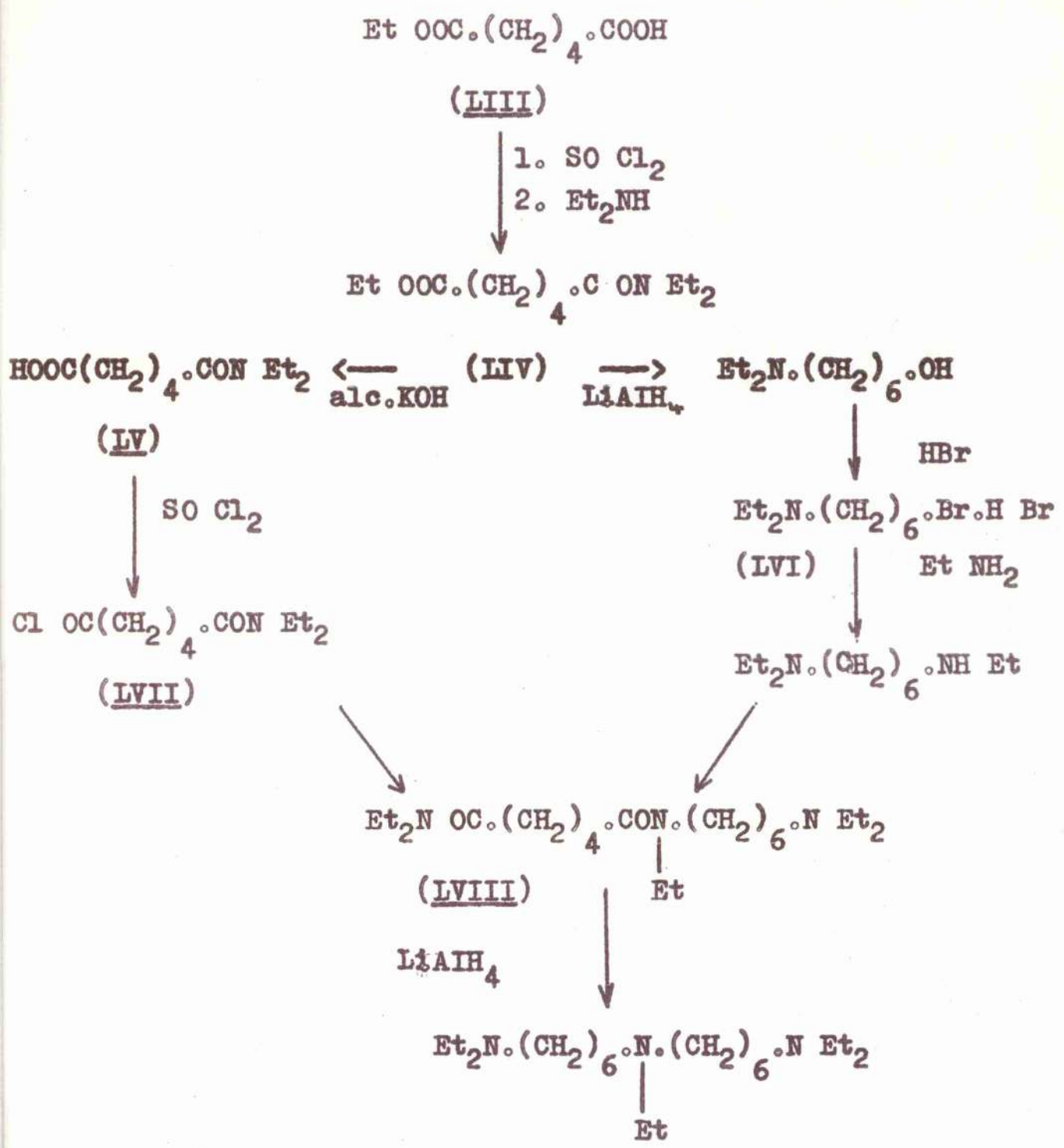
When 6-bromohexyldiethylamine (XLVIII) was refluxed with excess ethylamine for two hours the main product was 6-diethylaminohexylethylamine (XLIX) and only a little of the triamine (L) was obtained. The reaction of 6-bromohexyldiethylamine with 6-diethylaminohexylethylamine also gave poor yields of the triamine due to the instability of 6-bromohexyldiethylamine which readily cyclised on heating to give 1,1-diethyl-1-azacycloheptylinium bromide (LII).

$Br^{\ominus}$

(LII)

6-Chlorohexyldiethylamine, although less active than the bromo compound, was much more stable and could be condensed with 6-diethylaminohexylethylamine by refluxing in xylene for five hours to give improved yields (19%) of bis(6-diethylaminohexyl)ethylamine.

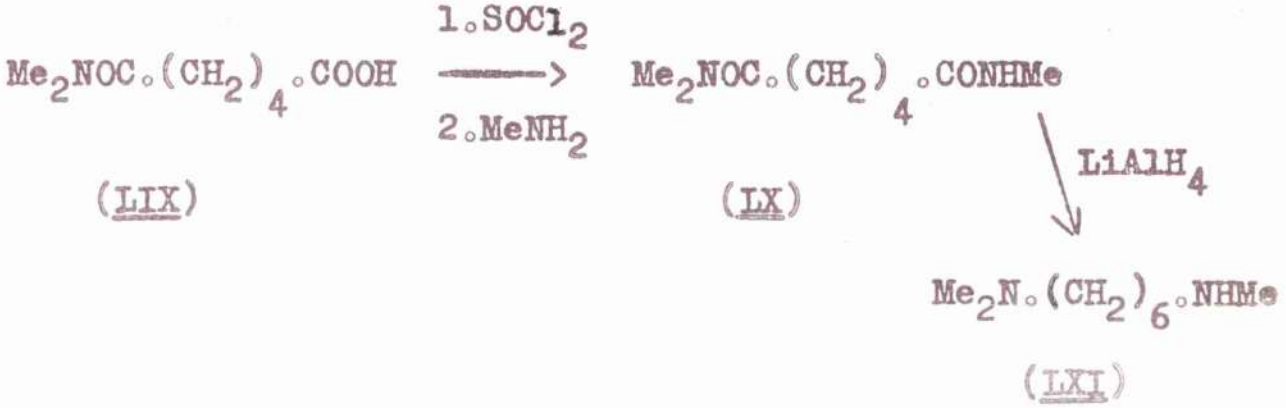
In the later work of Carey, Edwards, Lewis and Stenlake[37] an alternative method was devised for the synthesis of the triamine in greater yield from readily available starting materials.



The route to 6-hydroxyhexyldiethylamine from ethyl hydrogen adipate (LIII) was based on the synthesis of 6-hydroxyhexyldimethylamine by Andrews, Bergel and Morrison.[117] Hydrolysis of ethyl N N - diethyladipamate (LIV) gave N N - diethyladipamic

acid (LV). Esterification of 6-hydroxyhexyldiethylamine with hydrobromic acid (48%) gave 6-bromohexyldiethylamine hydrobromide (LVI) which with excess ethylamine yielded 6-diethylaminohexylethylamine. The use of the hydrobromide rather than the corresponding base prevented cyclisation during subsequent reactions and led to increased yields. N N - Diethyladipamoyl chloride (LVII) obtained by refluxing N N - diethyladipamic acid with thionyl chloride in benzene was condensed with 6-diethylaminohexylethylamine to give N - diethylaminohexyl - N' N" N" - triethyladipamide (LVIII) which was reduced with lithium aluminium hydride to give the triamine. This route was also employed for the synthesis of the corresponding n-propyl triamine.

However in the course of the present work it was found that 6-hydroxyhexyldimethylamine was only partially esterified after five hours refluxing with hydrobromic acid. The facile preparation of N N' N' - trimethyladipamide (LX) from N N - dimethyladipamic acid (LIX) and its subsequent reduction with lithium aluminium hydride to 6-dimethylaminohexylmethylamine (LXI) suggested the modified route adopted for the synthesis of all the diamines used in the present series.



(LIX) (LX) (LXI)

Ethyl hydrogen adipate, the key intermediate for the preparation of N N - disubstituted adipamic acids was prepared by Edwards' modification of the method described by Swann, Oehler and Buswell [118] The modified synthesis is simpler and quicker but gives lower yields (60 vs.71-75%) than the original method. Edwards collected diethyl adipate and ethyl hydrogen adipate as one fraction from the distillation of the reaction mixture, isolating the product by solvent extraction. However, it was possible to separate the greater proportion of the product by fractional distillation using a short, lagged column. Under these conditions only a little ethyl hydrogen adipate was recovered from the diethyl adipate.

Ethyl hydrogen adipate treated with thionyl chloride on the water bath for $1^1/2$ hr. gave the acid chloride which with excess of the secondary amine in ether formed the amido-ester. Alkaline hydrolysis of the ester, as described by Carey, Edwards, Lewis and Stenlake, [37] yielded the amidoacid. Refluxing for one hour on the water bath with a slight excess of ethanolic potassium hydroxide (approx. 2/3 N) was adequate for the saponification of ethyl N N - dimethyladipamate and ethyl N N - di-n-butyladipamate, but ethyl piperidinoadipamate was only partially (ca.50%) saponified. The reflux time was therefore extended to three

hours and, as this was quite satisfactory, the other heterocyclic esters were similarly treated. In all cases the solution was adjusted to pH 7 by the addition of dilute hydrochloric acid to prevent alkaline hydrolysis of the amide link during the evaporation of the ethanol.

N N - Dimethyl -, piperidino - and morpholino - adipamic acids were all freely soluble in cold water. It was necessary therefore to keep the volume of the aqueous acid solution to a minimum during the extraction, if satisfactory yields were to be obtained.

N N - Dimethyladipamic acid was sparingly soluble in all common organic solvents and could only be isolated by continuous extraction. Unexpectedly, ether was an excellent solvent by this method giving an almost pure product. Prelog[119] has previously prepared this acid from the dimethylammonium salt of adipic acid but he could not isolate it from the reaction mixture. N N - Dimethyladipamic acid underwent disproportionation during distillation (vide supra) and not until several months later was it successfully distilled. The product obtained from the continuous extraction was not further purified for the preparation of N N N' - trimethyladipamide.

N N - Di-n-butyladipamic acid, although much more soluble in organic solvents than the methyl acid was obtained

in better yield by continuous extraction. This acid also disproportionated on distillation, but this difficulty was overcome by omitting the leak (vide Supra) and the acid was always distilled before further use. The acids of the heterocyclic series were readily extracted from aqueous acid solution with chloroform, but were usually contaminated with a little adipic acid which could be detected by an increase in the melting point of the product and its incomplete solubility in cold water. Adipic acid is insoluble in benzene[120] and could be completely removed by filtration.

DISPROPORTIONATION OF AMIDOACIDS.

On every occasion N N - dimethyladipamic acid disproportionated during distillation giving a product, b.p. 136 - 154°/0.1 mm., with a very high equivalent. This was found to consist largely of N N N'N' - tetramethyladipamide, but the other products were not identified. The distillation was characterised by the necessity for prolonged heating and by a considerable amount of charring before distillation occurred. N N - Di-n-butyladipamic acid was distilled twice without incident, but on the third occasion disproportionated. Three fractions totalling only about half of the original 33 grammes of acid were obtained, the remainder of the material being completely charred. The first fraction was

a crystalline material, insoluble in benzene, and proved to be adipic acid (ca.1.2 g.). A basic separation of the other two fractions which were collected together gave N N - di-n-butyladipamic acid (ca. 7g.) and N N N'N' - tetra-n-butyladipamic acid (ca. 8g.). Thus these three products accounted for almost all the distillate.

The only difference between the three distillations appeared to be that orange sticks had been used on the first two occasions while on the third a leak was used. To confirm this the recovered acid was redistilled using an orange stick, and no disproportionation occurred. All subsequent distillations with an orange stick were also satisfactory. A sample of N N - dimethyladipamic acid (5 g.) was similarly distilled and the pure product obtained as a golden yellow oil which slowly crystallised.

Since Carey, Edwards, Lewis and Stenlake[37] had previously found no evidence of disproportionation with N N - diethyl - and N N - di-n-propyl- adipamic acids, these were re-examined under the conditions described above for the n-butyl acid, but the findings of these workers were confirmed.

The literature contains few references to this type of disproportionation, or transacylation. Edwards, Lewis, McPhail, Muir and Stenlake have reported[113] a similar occurrence with N N - diethyldiglycollamic acid which

appears to disproportionate even more readily than the adipamic acids. When Prelog[119] originally prepared N N - dimethyladipamic and also N N - dimethylsuccinic acids from their mono-dimethylammonium salts he found that the distillates contained appreciable proportions of the free acids or their anhydrides, and N N N'N' - tetramethyldiamides. However their origin is doubtful since they may have been formed by dissociation of the salt. Cherbuliez and Landolt[121] have reported the preparation of formamide, acetamide, benzamide and nicotinamide in good yields by heating the appropriate acid with urea at temperatures ranging from 140 to 230°. They also prepared one disubstituted amide, diethylnicotinamide, by heating nicotinic acid and tetraethylurea at 220 - 280° for four hours. An acidolysis reaction was proposed.

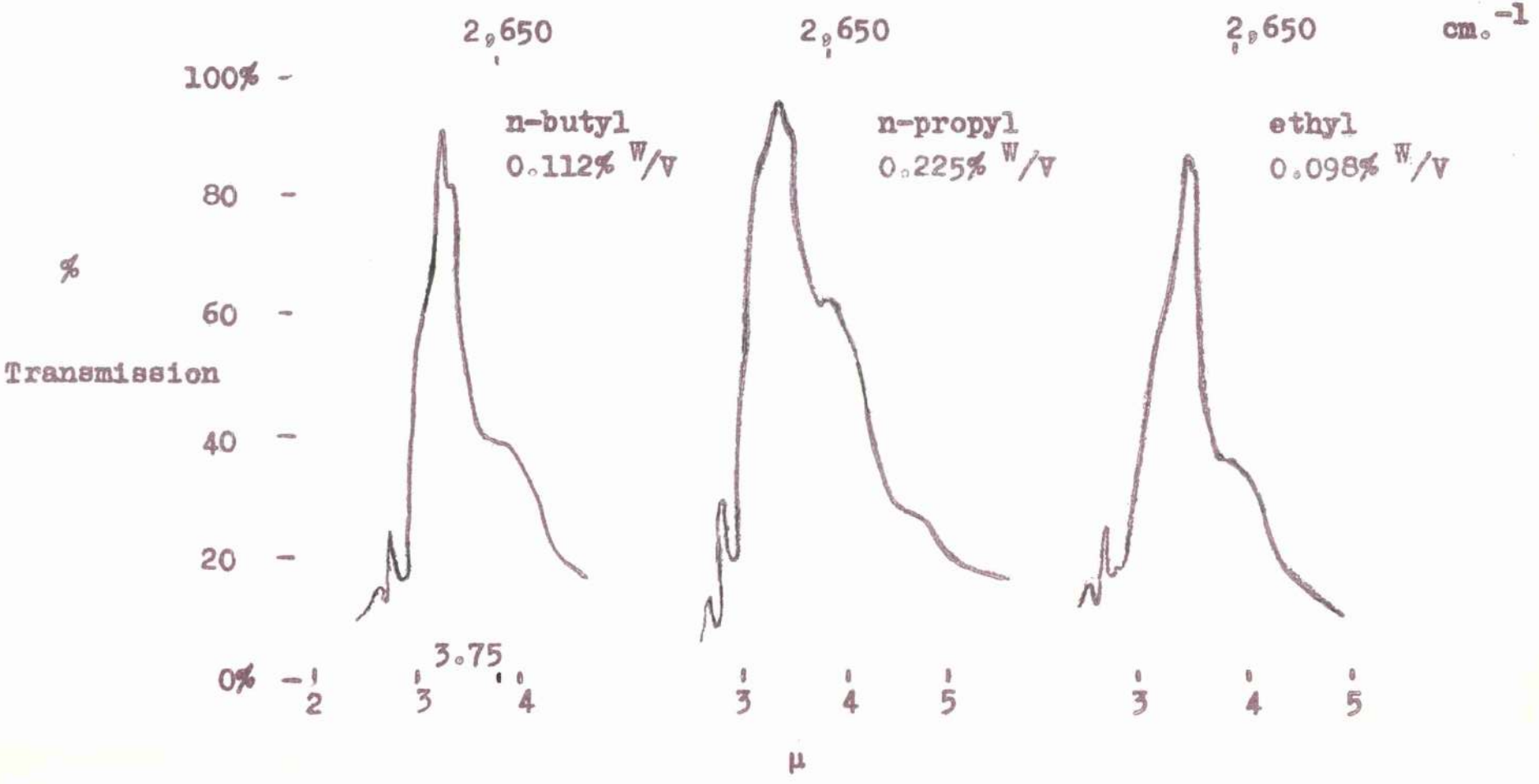
The exchange of acyl groups in N - acylated amines, aminoacids and peptides by the action of organic acids has also been reported. Acetanilide heated for 8 to 14 hours at 120 to 130° with dry trichloroacetic acid yielded 40 to 80% of N - phenyltrichloroacetamide.[122]

The influence of air on the disproportionation of N N - di-n-butyladipamic acid and the reversibility of the reaction were examined in two simple experiments. Two approximately equal samples of acid, one in an open ampoule

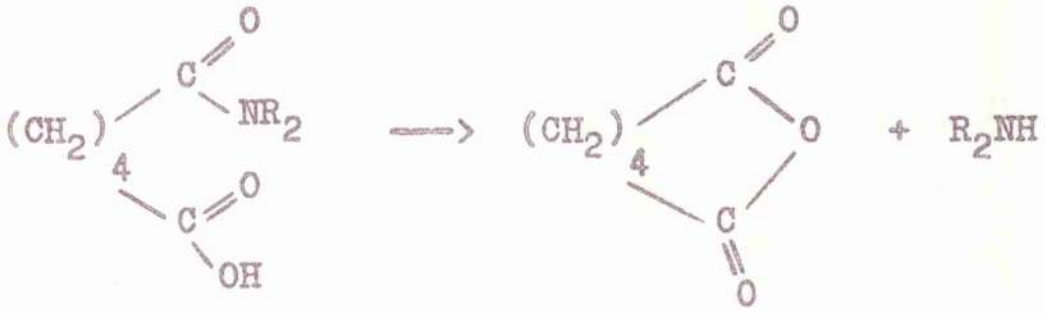
and the other in a sealed ampoule filled with nitrogen, were heated for one hour at 250°. Since both samples disproportionated to the same extent it appears that the duration and the temperature of heating may be more important factors than the presence of air. Cherbuliez and Landolt[121] have stated that acidolysis reactions are slow, hence both temperature and time would be important. This might explain why disproportionation was not observed in all four adipamic acids.

The reversibility of the reaction and its dependence on air was examined by heating two samples of an equimolecular mixture of adipic acid and N N N'N' - tetra-n-butyladipamide at 150° for 1 1/2 hours, air being drawn through one sample and nitrogen through the other. However no amidoacid was formed. This would seem to indicate an intramolecular mechanism ; possibly as shown in the scheme, although no free amine was detected during the disproportionation.

Fig.4. Hydroxyl band of I-R spectra of N N - dialkyladipamic acids in CCl_4, 1 cm. Quartz cells, path length 10 mm., temp. 25°C. Intramolecular hydrogen bonding sub-maxima occurring at 3.75μ.(160)

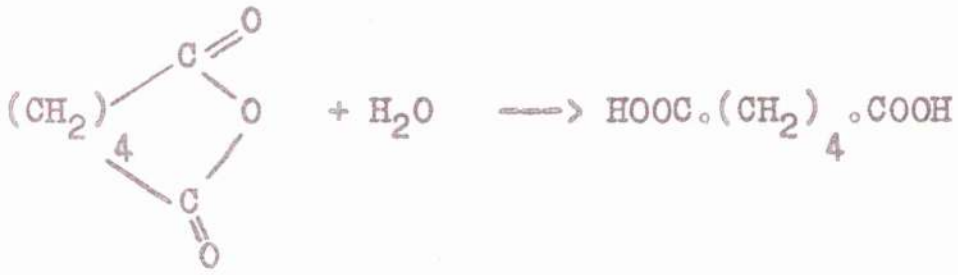


Intramolecular anhydride formation

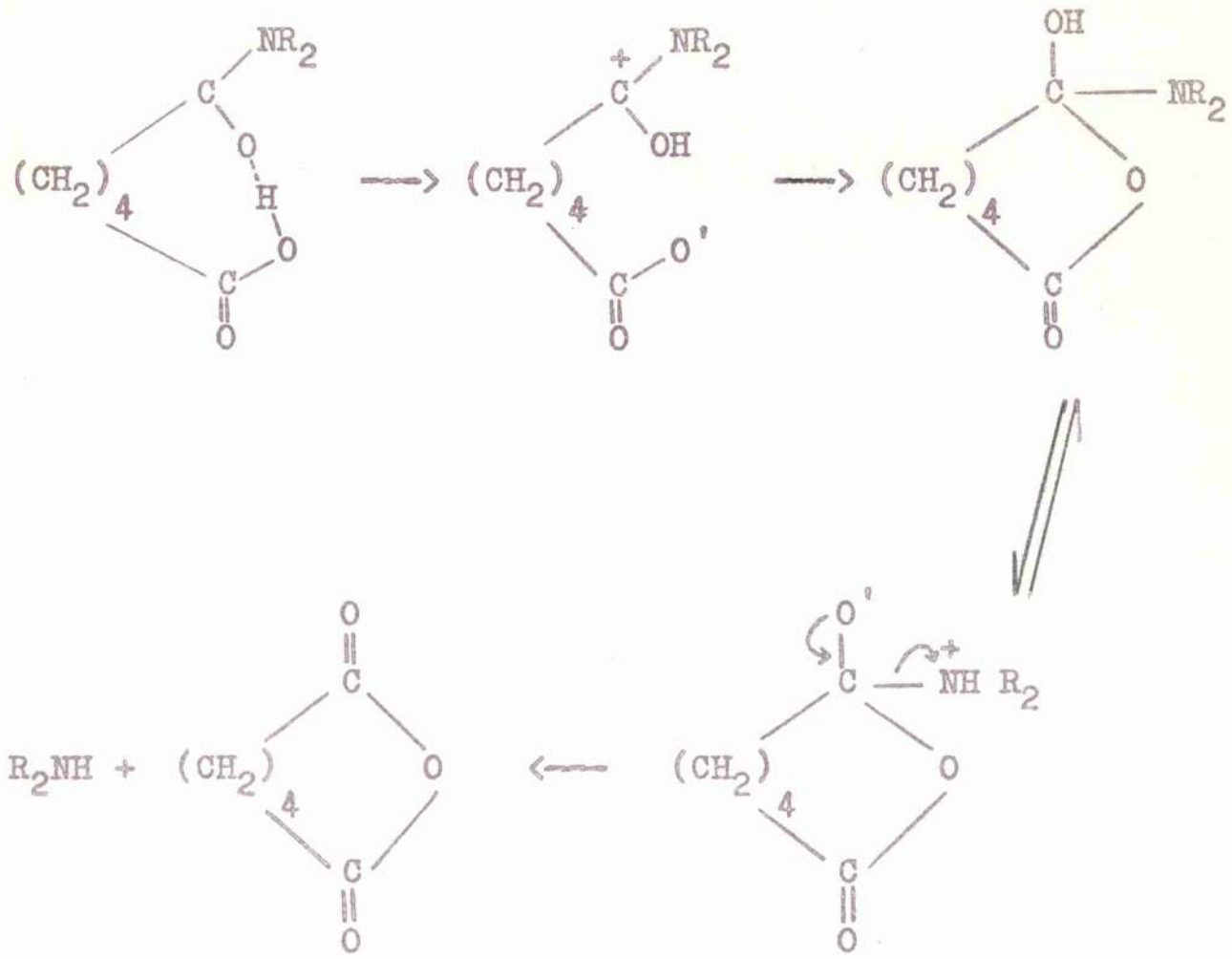
1. 

2. $R_2N\,OC.(CH_2)_4.COOH + R_2NH \longrightarrow R_2NOC.(CH_2)_4.COONH_2R_2$

3. $R_2N\,OC.(CH_2)_4.COONH_2R_2 \longrightarrow R_2N\,OC.(CH_2)_4.CONR_2 + H_2O$

4. 

Adipic acid can form both mono- and poly- molecular anhydrides.[123] The N N - di-n-butylammonium salt formed from the liberated amine and the amidoacid in step 2 could, by the loss of water, produce the diamide and adipic acid in steps 3 and 4 respectively. Supporting a mechanism of this type is the fact that infra-red spectra of the N N - dialkyl-adipamic acids reveals the presence of intramolecular hydrogen bonding, Figure 4. The transfer of a proton could facilitate anhydride formation :-



However, since all four adipamic acids exhibit hydrogen bonding, this mechanism does not explain their different behaviour and it would appear that other factors must be involved. A free radical step seemed to be indicated when N N - di-n-butyladipamic acid distilled with a leak did not disproportionate when a free radical inhibitor was present.

Preparation of diamides.

The diamides were prepared by the condensation of the appropriate adipamoyl chloride with excess primary amine in benzene. In contrast to ethyl hydrogen adipate

the amidoacids were extremely reactive towards thionyl chloride. Excess reagent and solvent were removed under reduced pressure when the brisk evolution of hydrogen chloride subsided, and more benzene was added and evaporated to remove any traces of thionyl chloride. The products were invariably dark viscous oils which, with the exception of N N - dimethyladipamoyl chloride, were freely soluble in benzene.

The presence of a good excess of the amine during the condensation prevented any formation of the bis compound (LXII).

(LXII)

Amides of this type are very resistant to lithium aluminium reduction[113], so that despite their attraction as intermediates for the preparation of triamines their formation in the above reaction must be avoided. The precipitated amine hydrochloride was filtered off and the filtrate treated in one of two ways. For N N' N' - trimethyladipamide water was completely excluded since the product was too water-soluble. The filtrate was therefore dried over sodium sulphate, evaporated and the diamide

purified by fractional distillation or recrystallisation. In the preparation of N N'N' - tri-n-butyladipamide and the other diamides the filtrate was washed with dilute hydrochloric acid to remove excess amine, but an attempted alkaline washing to remove any unreacted N N - di-n-butyladipamic acid produced an emulsion which was only broken with difficulty. This also occurred with N - ethyladipamoylpiperidine and N - ethyladipamoylmorpholine and in subsequent experiments this step was omitted. Drying over a basic desiccant such as sodium carbonate was tried for N - ethyladipamoylpiperidine but was not successful. These diamides were therefore purified by fractional distillation, rejecting the first fraction if the infra-red spectrum showed an acid carbonyl band at about 1700 $cm.^{-1}$

Lithium aluminium hydride reduction of diamides.

With the exception of N N'N' - tri-n-butyladipamide all the diamides were sparingly soluble in ether, but freely soluble in tetrahydrofuran which has been recommended (124) as an alternative solvent for reductions with lithium aluminium hydride. There are, however, two disadvantages to the use of this solvent. Firstly it is very difficult to obtain completely dry and, secondly, its miscibility with water is undesirable during the extraction procedure since large volumes of sodium hydroxide solution are generally required to dissolve the aluminium hydroxide

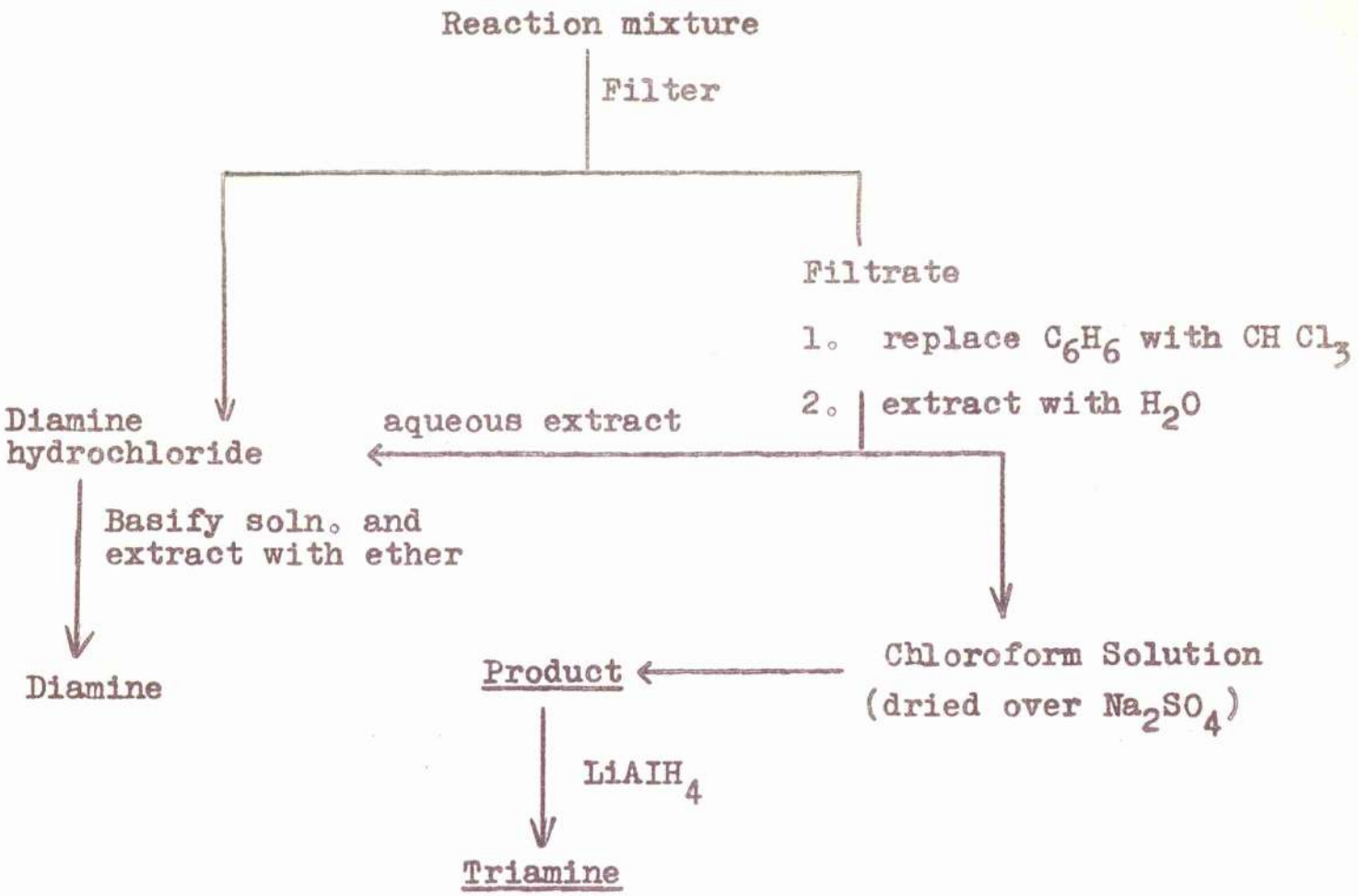
formed on decomposition of the complex.[124] However, the latter difficulty was overcome by the decomposition procedure of Amundsen and Nelson.[125] By this method the excess reagent and the complex were decomposed by the successive addition of n, 3/4 n and 3.5 n mls. of water, sodium hydroxide (20%) and water, where n equals the number of grammes of lithium aluminium hydride used. This gave a granular precipitate which could be very readily washed with solvent. Thus in the later reductions of ether-insoluble compounds the use of ether-tetrahydrofuran mixtures together with this method of decomposition gave very satisfactory results.

Preparation of triamines.

The crude adipamoyl chlorides were refluxed with excess diamine in benzene and the condensation products reduced with lithium aluminium hydride in ether to give the triamine. Carey, Edwards, Stenlake and Zoha[37] extracted the basic material from the condensation reaction with hydrochloric acid (10%), basified this extract and recovered the diamine and the condensation product by extracting with ether. Most of the diamine was removed by distillation before the final reduction. This procedure was satisfactory for the isolation of N - dimethylaminohexyl - N' N" N" - trimethyladipamide, but N - di-n-butylaminohexyl - N' N" N" - tri-n-butyladipamide could not be extracted from the reaction mixture with

hydrochloric acid. Only N N - di-n-butylaminohexyl-n-butylamine was obtained. Evaporation of the reaction mixture gave the product as a viscous black oil.

This finding led to the modified method of isolation outlined below for the heterocyclic compounds.



The precipitated diamine hydrochloride was filtered off from the reaction mixture and the benzene evaporated from the filtrate and replaced with chloroform which was the

better solvent. This solution was washed with water to remove any diamine hydrochloride and the aqueous extract used to dissolve the precipitated hydrochloride from which the diamine was recovered by basifying and extracting with ether. The chloroform solution was dried over sodium sulphate, evaporated and the product reduced to the triamine. Unreacted amidoacid was reduced to the amino-alcohol and removed during the fractional distillation of the triamine, being detected by the hydroxyl peak at 3,400 $cm.^{-1}$ in the infra-red spectrum.

Quaternisation of triamines.

The triamine was generally treated with three times its weight of alkyl halide with or without a solvent. Bis(6-dimethylaminohexyl)methylamine quaternised in ethanol with both methyl iodide and ethyl iodide to give crisp, white solids which recrystallised readily. Bis(6-di-n-butylaminohexyl)n-butylamine reacted slowly with alkyl halides and a solvent was used only in the preparation of the methiodide. The quaternary salts were hygroscopic , and both the ethiodide and n-propiodide were obtained as oils which solidified on trituration with ether. Only the methiodide could be recrystallised; the other three salts were purified by precipitation with dry ether.

Bis(6-piperidinohexyl)ethylamine quaternised readily with methyl iodide in ethanol, and the salt could be recrystallised and collected on a filter. However, the ethiodide was too hygroscopic to collect by filtration and was therefore centrifuged. The supernatant mother liquor was decanted and the crystalline quaternary salt washed by centrifuging with three successive portions of dry ether, and dried in a vacuum desiccator. The propiodide could not be prepared analytically pure, since it appeared to decompose on drying *in vacuo*. This method was also used for the hygroscopic salts of the morpholine base. Gladych and Taylor[87] have used a similar procedure for the hygroscopic quaternary salts of some tetrahydropapaverine derivatives.

Trichloroacetic acid has been reported[126] to form highly crystalline trichloroacetates of general formula (LXIII)

$$(R_4\overset{+}{N}\ Cl_3C.COO^{-})\ Cl_3C.COOH$$

(LXIII)

from hygroscopic mono - and bis - quaternary halides. However both the piperidinium triethiodide and the morpholinium trimethiodide gave oily products which solidified only after prolonged trituration.

SYNTHESES IN THE TETRAHYDROPAPAVERINE SERIES .

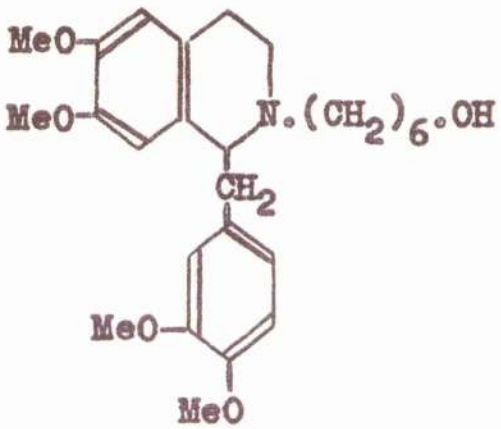
Since tetrahydropapaverine is a high-boiling viscous oil it was decided not to attempt the distillation of this series of compounds, but to purify them by solvent extraction or chromatography wherever possible. Tetrahydropapaverine was obtained from the hydriodide as described by Pyman,[127] but was mostly prepared by the high pressure hydrogenation of papaverine[128] using a Raney nickel catalyst prepared according to Vogel.[129] Späth and Burger[130] and Corrodi and Hardegger[116] have also obtained good yields (60%) by the electrolytic reduction of papaverine, while Pyman has reported[127] yields of 39% from the reduction of papaverine with tin and hydrochloric acid.

As only limited amounts of tetrahydropapaverine were available triethylamine was used as an acid-acceptor in the preparation of ethyl-2-tetrahydropapaverinyladipamate and bis(6,2'-tetrahydropapaverinylhexyl)ethylamine. 2-Tetrahydropapaverinyladipamic acid was prepared from the ester as described for piperidinoadipamic acid and small amounts of adipic acid were removed from the product in the usual manner. The acid was a hygroscopic solid which could not be recrystallised since it liquified in contact with solvents. In the preparation of N - ethyladipamoyl-tetrahydropapaverine the reaction mixture was extracted

with sodium carbonate solution to remove any unreacted acid and the product was obtained as a very hygroscopic solid which showed no acid carbonyl band in the infra-red spectrum. Lithium aluminium hydride reduction of the diamide in a mixture of ether and tetrahydrofuran (1 : 1) gave 6, 2'-tetrahydropapaverinylhexylethylamine. The diamine had a high equivalent but showed only one well defined spot on a paper chromatogram indicating that the impurity was non-basic, probably starting material. Passage through an alumina column gave a cleaner product with an unchanged R_f value but a better equivalent. Attempted recrystallisation of the reineckate from acetone, water and ethanol gave an oil which solidified on triturating and analysed satisfactorily.

A slight excess of the amidoacid was used in the preparation of bis(6,2'- tetrahydropapaverinylhexyl)ethylamine since it was assumed that unreacted acid could be removed from the condensation reaction mixture, whereas any excess diamine would be very difficult to separate from the triamine. There was little or no emulsion formation during the alkaline extraction. Reduction of the condensation product gave the triamine as a viscous yellow oil. This had a high equivalent and the paper chromatogram showed extensive tailing. Chromatography on an alumina column

gave the pure triamine and a much smaller amount of a yellow solid which analysed for 6-hydroxylhexyl-2' - tetrahydropapaverine (LXIV).



(LXIV)

The methiodide of bis(6,2' - tetrahydropapaverinylhexyl) ethylamine was readily prepared but the ethiodide generally decomposed to a brown oil.

A table of all the quaternary salts prepared is included with the pharmacological results in a later section.

EXPERIMENTAL

Melting points are uncorrected.

The author wishes to thank Dr. A.C. Syme, and Mr. W. McCorkindale of the Chemistry Department and Miss M. Buchanan and Mr. D. Caldwell of the Pharmacy Department for carrying out the micro-analysis.

PREPARATION OF STARTING MATERIAL.

Ethyl hydrogen adipate.

Diethyl adipate (123g.), adipic acid (153g.) and hydrochloric acid (27 ml.) were heated to 160 - 170° with stirring until the solution was homogeneous. The temperature was allowed to fall to 120 - 130°, ethanol (65 ml.) was added, and the temperature of the mixture maintained at 130 - 140° for a further two hours. The ethanol and the hydrochloric acid were then removed under reduced pressure and the residual mixture fractionally distilled using a short (three plates) lagged column. Diethyl adipate was recovered over the range 88 - 114°/0.04 mm. using a water condenser which was turned off and lagged when the ethyl hydrogen adipate began to crystallise on the inner surface, b.p.116 - 124°/0.04 mm. The distillation was stopped when the vapour temperature started to rise fairly rapidly. The diethyl adipate was dissolved in ether and extracted with a concentrated solution of sodium carbonate which was in turn acidified with dilute hydrochloric acid, extracted with ether and the ethereal solution dried (Na_2SO_4). Removal of the solvent gave ethyl hydrogen adipate which was combined with the bulk fraction and redistilled as before, (109 g., 60%), b.p. 136 - 140°/0.7 mm., m.p. 28°.

Found: equiv. (titn.) 173.6. Calc. for $C_8H_{14}O_4$: 174.2.

Literature[118] m.p. 28°.

PREPARATION OF BIS(6-DIMETHYLAMINOHEXYL-METHYLAMINE.

Ethyl N N - dimethyladipamate.

Ethyl hydrogen adipate (40g.) was refluxed with excess thionyl chloride (50 ml.) for $1^1/2$ hr. at 90 - 100°. Excess thionyl chloride was removed under reduced pressure, and successive portions of dry ether (2 x 10 ml.) added and evaporated to remove any residual reagent. The crude acid chloride in dry ether (50 ml.) was slowly added to a stirred solution of anhydrous dimethylamine (35g.) in dry ether (300 ml.), maintained at 0° by external cooling. At the end of the addition the reaction mixture was refluxed for one hour. The dimethylamine hydrochloride precipitated during the reaction was filtered off, washed with more ether and the filtrate and washings were extracted with water then with aqueous sodium carbonate and finally dried (Na_2SO_4). Removal of the ether and distillation gave the required product as a pale yellow oil, (36.5g., 79%), b.p. 128°/ 1.5 mm., n_D^{24} 1.4560. Andrews, Bergel and Morrison[117] found b.p. 102 - 106°/0.25 mm., n_D^{20} 1.4573.

Found: N, 7.0. Calc. for $C_{10}H_{19}NO_3$: N, 7.0%.

The following amide esters were similarly prepared from ethyl hydrogen adipate, except that the acid chloride was added to the reaction vessel at room temperature and, during the extraction procedure the ethereal solution was first washed with dilute hydrochloric acid to remove excess of the secondary amine:

Ethyl N N- di-n-butyladipamate, yellow oil, (52 g., 93%), b.p. 146°/0.1 mm., n_D^{16} 1.4580. Johnson[131] quotes b.p. 136-138°/0.15 mm., n_D^{20} 1.4569

Found: N, 4.9. Calc. for $C_{16}H_{31}NO_3$: N, 4.9%.

Ethyl piperidinoadipamate, yellow oil, (60.5 g., 78%), b.p. 166-167°/0.2 mm. Avison[132] gives b.p. 148-152°/0.5 mm.

Found: N, 6.0. Calc. for $C_{13}H_{23}NO_3$: N, 5.8%.

Ethyl morpholinoadipamate, pale yellow oil, (52 g., 71%), b.p. 177-183°/0.1 mm.

Found: N, 5.8. $C_{12}H_{21}NO_4$ requires N, 5.8%.

REACTIONS WITH 6-HYDROXYHEXYLDIMETHYLAMINE.

6 - Hydroxyhexyldimethylamine.

Ethyl N N - dimethyladipamate (21 g.) in dry ether (50 ml.) was added slowly to a stirred, refluxing suspension of lithium aluminium hydride (5 g.) in dry ether (120 ml.) and the solution was refluxed for five hours. The reaction mixture was cooled externally in an ice bath and brine added cautiously to decompose the complex and excess lithium aluminium hydride. Sodium hydroxide (100 ml., 20%) was added with stirring to produce a gel from which the ethereal supernatant was decanted. The gel was extracted with more ether (2 x 200 ml.) and the combined ether extracts dried (Na_2SO_4). Evaporation of the solvent yielded a mobile

liquid which was distilled to give the product as a colourless oil, (10 g., 66%), b.p. 70°/5 mm., n_D^{20} 1.4485. Andrews, Bergel and Morrison[117] give b.p. 114 - 116°/12 mm., $n_D^{20.5}$ 1.4482.

Found: N, 9.5; equiv. (titn.) 148.3.

Calc. for $C_8 H_{19} N O$: 9.65% ; equiv. 145.2.

The aminoalcohol was exceedingly hygroscopic. The hydrochloride could be prepared as fine white needles by passing dry hydrogen chloride into an ethereal solution of the base, but the crystals were deliquescent.

6 - Hydroxyhexyldimethylamine methiodide.

Methyl iodide (1 ml) was slowly added to the base (0.6 g.) in ether (4 ml.). Recrystallisation of the dense white precipitate (ethanol-ether) gave a quantitative yield of the product as white leaflets, m.p. 126°.

Found: N, 4.9; I, 44.4.

C_9H_{22} I N O requires N, 4.9 ; I, 44.25%.

Hydrobromide, recrystallised from ethanol-ether as white leaflets, m.p. 85 - 88°.

Found: N, 6.0 ; Br, 35.4. $C_8 H_{20}$ Br N O requires N, 6.2 ; Br, 35.8%.

Attempted preparation of 6 - dimethylaminohexylmethylamine

6 - Hydroxyhexyldimethylamine (4.3 g.) was treated with hydrobromic acid (10 ml., 48%) at 90° for 5 hr. The reaction mixture was concentrated to a thick syrup under reduced pressure and the last traces of hydrobromic acid removed by the successive addition and removal under reduced

pressure of water and ethanol. A steady stream of methylamine was passed into a stirred refluxing solution of the residual oil in ethanol (125 ml.) for $1^{3}/4$ hr. The ethanol was distilled off leaving a damp, crystalline mass which was basified with sodium hydroxide (20%) and extracted with ether. After drying (Na_2SO_4) and removal of the ether a pale yellow oil was obtained. Fractional distillation gave a colourless base (1.6 g.), b.p. 85°/0.1 mm. This material had equivalent (titn.) 136.3 vs. 79.1 required for 6 - dimethylaminohexylmethylamine. The infra-red spectrum was almost identical to that of 6 - hydroxyhexyldimethylamine and paper chromatography (Watmans No.1) by the ascending method using butanol -acetic acid - water (4 : 1 : 5) gave a very dense spot corresponding to the aminoalcohol and a paler spot of R_f 0.27 which was probably the required diamine.

N N - Dimethyladipamic acid.

Ethyl N N - dimethyladipamate (12.5 g.) was refluxed for 1 hr. with ethanolic potassium hydroxide (approx. $^{2}/3$ N; 135 ml.), the solution cooled and just neutralised with dilute hydrochloric acid. The ethanol was removed under reduced pressure and benzene (2 x 50 ml.) added and similarly evaporated to remove the last traces of ethanol. The residual potassium salt was acidified with hydrochloric acid (20 ml., 25%) and the precipitated potassium chloride filtered off. Continuous extraction of the filtrate with ether gave the required acid as an oil which settled out from the ether in

the reservoir. Evaporation of the solvent left a viscous grey oil which was not distilled (8.2 g., 76%).

Found: N, 7.9 ; equiv. (titn.) 175.6 .

$C_8 H_{15} N O_3$ requires N, 8.1 ; equiv. 173.2.

Distillation of the acid (vide supra) gave a yellow oil (b.p. 178°/0.05 mm., equiv. 172.2) which crystallised as rosettes of thick white needles after several months.

N N N' - Trimethyladipamide.

Thionyl chloride (8 ml.) in benzene (30 ml.) was added to N N - dimethyladipamic acid (10 g.) suspended in benzene (70 ml.) and the excess reagent and the solvent were removed almost immediately at below 50° under reduced pressure. Another portion of benzene (20 ml.) was added and evaporated. The sparingly soluble crude N N - dimethyladipamoyl chloride suspended in benzene (50 ml.) was stirred at 0° and dry methylamine passed in for two hours until the uptake was complete. The precipitated methylamine hydrochloride was filtered off, washed with benzene and the combined benzene extracts evaporated to dryness leaving a yellow oil (10.5 g., 98%) which crystallised rapidly. The product could be purified by recrystallisation (dry acetone - ether) as fine colourless needles, m.p. 54 - 56° which were collected under nitrogen, or by fractional distillation, b.p. 218 - 220°/0.2mm.

Found: N, 14.8. $C_9 H_{18} N_2 O_2$ requires N, 15.0%.

The diamide was extremely hygroscopic and almost insoluble in ether.

6 - Dimethylaminohexylmethylamine.

N N N' - trimethyladipamide (6.4 g.) in dry tetrahydrofuran (30 ml.) was added over a period of 25 min. to a stirred refluxing suspension of excess lithium aluminium hydride (5 g.) in tetrahydrofuran (60 ml.). Refluxing was continued for five hours and the excess reagent decomposed with water. The supernatant tetrahydrofuran was decanted and the gel extracted with several further volumes of tetrahydrofuran (250 ml.). The combined extracts were dried (Na_2SO_4), the solvent removed and the product distilled to give 6 - dimethylaminohexylmethylamine, as a colourless, mobile oil, (3.0 g., 55%), b.p. 78°/0.2 mm.

Found: N, 17.4 ; equiv. (titn.) 79.2.

$C_9 H_{22} N_2$ requires N, 17.7% ; equiv. 79.1.

Bis (6 - dimethylaminohexyl)methylamine .

N N - Dimethyladipamic acid (2.9 g.) was treated with thionyl chloride (1.8 ml.) as previously described, and a suspension of the acid chloride in benzene (18 ml.) was added slowly to a stirred, refluxing solution of 6 - dimethylaminohexylmethylamine (5.5 g.) in benzene (35 ml.). After refluxing for a further 30 min. the reaction mixture was extracted with dilute hydrochloric acid (2 x 25 ml.) the acid solution basified with sodium hydroxide (35 ml., 20%), and extracted with benzene (4 x 50 ml.). The benzene solution was dried (Na_2SO_4) and the brown mobile oil (6.8 g.) which remained after evaporation of the solvent gave, on distillation, some 6 - dimethylaminohexylmethylamine (ca. 0.75 g.),

b.p. 58-60°/0.1 mm. The crude undistilled N - dimethylaminohexyl - N'N"N" - trimethyladipamide in ether was reduced with lithium aluminium hydride (1.5 g.) and the product extracted as described for 6-hydroxyhexyldimethylamine. Fractional distillation gave a forerun of the diamine (1.8 g.), and bis(6 - dimethylaminohexyl)methylamine, as a pale yellow oil (2.3 g., 48%), b.p. 130°/0.07 mm., n_D^{20} 1.4533.

Found: N, 14.95 ; equiv.(titn.) 95.3.

$C_{17}H_{39}N_3$ requires N, 14.7% ; equiv. 95.2.

Quaternisation of bis(6-dimethylaminohexyl)methylamine. 7,7 - Dimethyl-7-azoniatridecylenebis(trimethylammonium) tri-iodide.

Methyl iodide (0.65 ml.) was added to the base (0.5 g.) in dry ethanol (4 ml.) at room temperature. The mixture was allowed to stand overnight before filtering. Recrystallisation of the precipitate (water-ethanol-ether) gave a white microcrystalline powder (1.05 g., 84%), m.p. 246-248°(decomp.).

Found: N, 5.8 ; I, 53.85.

$C_{20}H_{48}I_3N_3$ requires N, 5.9 ; I, 53.5%.

7-Ethyl-7-methyl-7-azoniatridecylenebis(dimethylethylammonium)tri-iodide.

Ethyl iodide (0.8 ml.) was added to the base (0.5 g.) in ethanol (4 ml.) and the mixture allowed to stand for four days at room temperature. The precipitate was recrystallised

(ethanol-methanol) to give the product as a fine white powder, (0.8 g., 61%), m.p. 188-190°.

Found: N, 5.6 ; I, 50.8.

$C_{23}H_{54}I_3N_3$ requires N, 5.6 ; I, 50.55%

PREPARATION OF BIS(6-Di-n-BUTYLAMINOHEXYL)n-BUTYLAMINE

N N - Di-n-butyladipamic acid.

A. The acid was prepared by saponification of ethyl N N-di-n-butyladipamate (50 g.), vide infra, as previously described for the preparation of N N - dimethyladipamic acid. Continuous extraction of the aqueous acid solution with ether, and distillation gave the required product, as a very viscous, yellow oil, (39.2 g., 87%), b.p. 198°/0.03 mm., $n_D^{16.5}$ 1.4720.

Found: N, 5.2 ; equiv.(titn.) 257.8.

$C_{14}H_{27}NO_3$ requires N, 5.4% ; equiv. 257.4.

B. Saponification of the amide ester (12 g.) followed by extraction of the aqueous solution with benzene gave only 8.0 g. (74%) of product, b.p. 193-195°/0.08 mm.

N N N' - Tri-n-butyladipamide

Crude N N-di-n-butyladipamoyl chloride was prepared by refluxing the acid (28 g.) and thionyl chloride (16.5ml.) in benzene (25 ml.) at 90 - 95° for 7 min. and isolated as described for N N - dimethyladipamoyl chloride. The acid chloride in benzene (75 ml.) was then added to an ice-cold solution of n-butylamine (25 ml.) in benzene (100 ml.) and

the solution refluxed for one hour during which a further 5 ml. of n-butylamine and 50 ml. of benzene was added. The reaction mixture was filtered and washed with dilute hydrochloric acid (20 ml.) and water(10 ml.) and dried over sodium sulphate. Evaporation of the solvent left a brown viscous oil which was fractionally distilled, rejecting the first fraction. The required product was a viscous brown oil, (26.5 g., 78%), b.p. 214°/0.04 mm.

Found: N, 9.0. $C_{18}H_{36}N_2O_2$ requires N, 9.0%.

6 - Di-n-butylaminohexyl-n-butylamine.

6 - Di-n-butylaminohexyl-n-butylamine was obtained as a pale yellow oil, (14.3 g., 79%) $n_D^{23.5}$ 1.4502, by the reduction of the diamide (20 g.) in ether with lithium aluminium hydride (5 g.), as described under the preparation of 6 - hydroxyhexyldimethylamine. During the distillation of the product frothing occurred at 100° and continued until just before the boiling point, 132°/0.08 mm.

Found: N, 9.8 ; equiv.(titn.) 143.1.

$C_{18}H_{40}N_2$ requires N, 9.85% ; equiv. 142.3.

Bis(6 - di-n-butylaminohexyl)n-butylamine.

N N - Di-n-butyladipamoyl chloride in benzene (25 ml.), prepared from the acid (6.9 g.) as previously described, was added (15 min.) to a stirred, refluxing solution of 6 - di-n-butylaminohexyl-n-butylamine (15 g.) in benzene (50 ml.) and refluxed for thirty minutes. A gel formed on cooling,

therefore the solution was reheated on the water bath and extracted as described for the preparation of bis(6 - dimethylaminohexyl)methylamine. On distillation 6 - di-n-butylaminohexyl-n-butylamine (5.6 g., b.p. 124°/0.03 mm.) was recovered, but almost no residue was left in the distillation flask.

Evaporation of the reaction mixture left a viscous residue which was dried *in vacuo* and reduced with lithium aluminium hydride (3.5 g.) in ether. The product was fractionally distilled and yielded the required base only, (5.2 g.), b.p. 214 - 218°/0.05 mm., with a fore-run (1.0 g.) which distilled slowly over the range 130-214°/0.05 mm.
Found: N, 8.6 ; equiv.(titn.) 162.4 ; fore-run equiv. 165.0
$C_{32}H_{69}N_3$ requires N, 8.5% ; equiv. 165.3.
The total yield was 47%.

Quaternisation of bis(6 - di-n-butylaminohexyl)n-butylamine. 7-Methyl-7-n-butyl-7-azoniatridecylenebis(di-n-butylmethyl-ammonium)tri-iodide.

Methyl iodide (0.7 ml.) was added to the base (0.5 g.) in ethanol (4 ml.) and the solution allowed to stand overnight, then refluxed for twenty minutes and evaporated to dryness under reduced pressure. A brittle yellow solid was obtained which was refluxed with acetone, filtered, and washed with petroleum ether (b.p. 40-60°). Recrystallisation (ethanol-ethyl acetate-ether) gave the product, as a microcrystalline powder, (0.8 g., 86%), m.p. 176-180°(decomp.)

Found: N, 4.5 ; I, 41.3.

$C_{35} H_{78} I_3 N_3$ requires N, 4.6 ; I, 41.3%.

7-Ethyl-7-n-butyl-7-azoniatridecylenebis(di-n-butylethyl-ammonium)tri-iodide.

The base (0.7 g.) was refluxed with ethyl iodide (1.1 ml.) for 15 min. Removal of the excess reagent left a hygroscopic oil which solidified on standing overnight in dry ether. Precipitation from n-propanol -ethyl acetate gave the quaternary salt as a white powder, (1.2 g., 88%), m.p. 163-166°(decomp.).

Found: N, 4.3 ; I, 39.6.

$C_{38} H_{84} I_3 N_3$ requires N, 4.4 ; I, 39.5%.

7-n-Butyl-n-propyl-7-azoniatridecylenebis(di-n-butyl-n-propylammonium)tri-iodide.

The base (0.5 g.) was refluxed with n-propyl iodide (1ml.) on a water bath for 80 min. Excess reagent was removed under reduced pressure leaving an oil which was triturated with ether until it solidified. Pure product was obtained by precipitation from n-propanol - ethyl acetate - ether, (0.65 g., 64%), m.p. 143-146°(decomp.)

Found: N, 4.05 ; I, 37.8.

$C_{41} H_{90} I_3 N_3$ requires N, 4.2 ; I, 37.9%.

7,7-Di-n-butyl-7-azoniatridecylenebis(tri-n-butylammonium) tri-iodide.

The base (0.5 g.) refluxed with n-butyl iodide (1 ml.) for 80 min. gave the product as a brittle solid which was precipitated from ethanol - acetone -ether as a white powder (0.75 g., 71%), m.p. 140-142° (decomp.).

Found: N, 4.0 ; I, 37.0

$C_{44} H_{96} I_3 N_3$ requires N, 4.0 ; I, 36.3%.

DISPROPORTIONATION OF N N - DIALKYLADIPAMIC ACIDS.

N N - Dimethyladipamic acid.

When N N - dimethyladipamic acid was distilled using a leak, the product was a yellow oil, b.p. 136-154°/0.1 mm., with a very high equivalent (>300). The oil partially solidified on cooling and, on extracting with a warm mixture of carbon tetrachloride and petroleum ether (b.p. 40-60°), gave a crystalline material which re-crystallised from acetone - petroleum ether (b.p. 40-60°) as white platelets, m.p. 80-82°. Prelog[119] quotes m.p. 85° for N N N'N'- tetramethyladipamide.

Found: N, 13.7. Calc. for $C_{10} H_{20} N_2 O_2$: N, 14.0%.

N N - Di-n-butyladipamic acid .

N N - Di-n-butyladipamic acid (35 g.) also disproportionated during distillation, giving what appeared to be two or three fractions, the first of which, b.p. 198°/0.4 mm.,

crystallised in the condenser, while the second was a viscous yellow oil which distilled over a range, b.p. 204-214°. The weight of the total distillate was 17 g. The first fraction was washed with benzene (50 ml.) and the residue recrystallised (acetone - ether) to give adipic acid (ca. 1.2 g.), m.p. 153°. Literature[120] m.p. 152°.
Found: equiv.(titn.) 74.1. Calc. for $C_6 H_{10} O_4$: 73.1.

The benzene solution was combined with the second fraction and washed with sodium hydroxide (20%). After drying (Na_2SO_4) and evaporation of the benzene a pale yellow oil remained (7 g.) which crystallised as fine needles, m.p. 43°. A little of this material was recrystallised with difficulty as long, colourless needles, m.p. 50°.
Found: N, 7.4. Calc. for $C_{22} H_{44} N_2 O_2$: N, 7.5%.
N N N' N' - Tetra-n-butyladipamide has been reported by Campbell and Tryon[133], but no m.p. recorded.

The alkaline wash was acidified with hydrochloric acid, filtered to remove sodium chloride and extracted with benzene. The benzene solution was dried (Na_2SO_4) and the benzene distilled off leaving N N - di-n-butyladipamic acid (7 g.) contaminated with adipic acid, equivalent (titn.) 242. The impure amidoacid was redistilled without disproportionating using an orange-stick in place of the leak. Several subsequent distillations of this acid were also without incident when an orange stick was used. N N - Dimethyladipamic acid (5 g.) distilled readily, b.p. 178°/0.05 mm., by this method and analysed satisfactorily.

N N - Diethyl - and N N - di-n-propyl-adipamic acids.

Two samples of these acids, prepared by Carey, Edwards, Lewis and Stenlake,[37] were distilled, using an orange-stick and then a leak, but no disproportionation was observed. The boiling points and equivalents of the samples distilled with a leak were :-

N N - diethyladipamic acid, b.p. 172-179°/0.1 mm., equiv.(titn.) 201.1. Calc. for $C_{10} H_{19} N O_3$: equiv. 201.3.

N N - di-n-propyladipamic acid, b.p. 201-204°/0.1 mm., equiv.(titn.) 229.2. Calc. for $C_{12} H_{23} N O_3$: equiv. 229.3.

Experiments on the disproportionation reaction.

A. The influence of air.

Nitrogen was blown into an ampoule (25 ml.) containing pure N N - di-n-butyladipamic acid (4.5 g.) for several minutes and the ampoule quickly sealed. This ampoule and an open control containing 3.85 grammes of acid were heated on a Woods metal bath at 250° for one hour. Although the control sample was much darker in colour, the refractive indices were identical (n_D^{22} 1.4720) and both samples had disproportionated to some extent. The contents of the test ampoule were washed on to a filter with benzene removing the adipic acid, and the filtrate was treated as earlier described for the separation of N N - di-n-butyladipamic acid and N N N' N' - tetra-n-butyladipamide. The weights of the impure products recovered are indicated below :-

Adipic acid	Amidoacid	Amide
0.43g.(m.p.133-138°)	1.8g.(equiv.259.2)	1.3g.(m.p.32°)

The control gave similar proportions of the three compounds.

B. Reversibility.

Recovered N N N'N' - tetra-n-butyladipamide (3.9 g., m.p.42°) and adipic acid (1.5 g.) were mixed and divided into two equal portions which were degassed in a vacuum desiccator for several hours. These samples in identical flasks were placed in an oil bath at 150° for $1\frac{1}{2}$ hr. and air drawn through one flask and nitrogen through the other. Both samples were extracted as described above (A.) but only the starting materials were recovered.

C. Distillation in the presence of quinhydrone.

N N - Di-n-butyladipamic acid (1.5 g.) was distilled with a leak in the presence of quinhydrone (0.15 g.). The distillate was a pale yellow oil, bath temp. 255 - 285°/ 0.15 mm., contaminated with a little sublimed quinhydrone. Found: equiv.(titn.) 258.4.

PREPARATION OF HETEROCYCLIC TRIAMINES .

Piperidinoadipamic acid .

Ethyl piperidinoadipamate (17 g., vide infra) was refluxed with ethanolic potassium hydroxide (250 ml., approx. 2/3 N) for three hours. The solution was neutralised and the ethanol removed under reduced pressure and also two additional volumes of benzene (2 x 25 ml.). The residue was acidified with hydrochloric acid (15 ml.) and water (10 ml.), filtered, and extracted with chloroform (100 ml.). After drying (Na_2SO_4) and evaporation of the chloroform a golden yellow oil was obtained which crystallised spontaneously as rosettes of long needles. These were dissolved in a small volume of benzene and filtered to remove any adipic acid. Evaporation of the benzene and recrystallisation (charcoal) from chloroform - ether - petroleum ether (b.p. 40-60°) gave pure product as colourless rosettes, (14.5 g., 96%), m.p. 81-83°.

Found: N, 6.7 ; equiv.(titn.) 212.9.

$C_{11}H_{19}N\,O_3$ requires N, 6.6% ; equiv. 213.3.

Morpholinoadipamic acid .

This acid was prepared similarly from the ester (20 g.). Recrystallisation (chloroform - ether - petroleum ether, b.p. 40-60°) gave rosettes of white needles, (14 g., 79%), m.p. 63-65°.

Found: N, 6.65 ; equiv.(titn.) 215.9.

$C_{10}H_{17}N\,O_4$ requires N, 6.5% ; equiv. 215.2.

N - Ethyladipamoylpiperidine .

Piperidinoadipamic acid (13 g.) in benzene (50 ml.) was treated with thionyl chloride (10 ml.) at 70-80° for 10 minutes, and the solvent and excess reagent, plus two additional volumes of benzene successively removed under reduced pressure. Anhydrous ethylamine (15 ml.) in benzene (20 ml.) was added with stirring to the crude acid chloride in benzene (80 ml.) at 0°. The reaction mixture was allowed to stand for several hours, filtered and evaporated, leaving a viscous black oil. A solution of this oil in chloroform (100 ml.) was washed with water (2 x 15 ml.) and dried (Na_2CO_3). Fraction distillation, as for N N N' - tri-n-butyladipamide, gave the required product, (10 g., 68%), b.p. 236-242°/0.13 mm.

Found: N, 11.1. $C_{13} H_{24} N_2 O_2$ requires N, 11.7%.

The diamide was a dark viscous oil which solidifed very slowly on standing.

N - Ethyladipamoylmorpholine, was similarly prepared from morpholinoadipamic acid (20.5 g.), but the acid was treated with thionyl chloride at 60-75° for 20 minutes.

Fractional distillation gave a light brown oil, (16.8 g., 73%), b.p. 234°/0.1 mm.

Found: N, 11.6. $C_{12} H_{22} N_2 O_3$ requires N, 11.6%.

The distillate crystallised quite rapidly on standing, m.p. 61-64° with softening at 56°.

6 - Piperidinohexylethylamine .

The diamide (22 g.) in a mixture of ether and tetrahydrofuran (100 ml., approx. 3:1) was slowly added to a refluxing suspension of lithium aluminium hydride in a similar mixture (100 ml.) and refluxed for five hours. Excess reagent was decomposed by the successive addition of water (8 ml.), sodium hydroxide (6 ml., 20%) and water (28 ml.) giving a granular precipitate which was readily extracted with ether. Fractional distillation gave the product as a mobile, colourless oil, (14.25 g., 73%), b.p. 112-118°/0.07 mm., n_D^{21} 1.4685.

Found: N, 12.7 ; equiv.(titn.) 109.3.

$C_{13}H_{28}N_2$ requires N, 13.5% ; equiv. 106.2.

Reineckate .

A little of the diamine in water acidified to congo red with dilute sulphuric acid gave an immediate precipitate on the addition of a saturated aqueous solution of ammonium reineckate. The precipitate was filtered off, washed with water and recrystallised from acetone - water as fine, pink platelets which were dried in vacuo below 50°.

The decomposition point was ca. 186°.

Found: N, 22.3 ; equiv.(titn.) 438.

$C_{21}H_{44}Cr_2N_{14}O_2S_8$ requires 22.15% ; equiv. 442.7.

6-Morpholinohexylethylamine was prepared similarly to 6-piperidinohexylethylamine from N-ethyladipamoylmorpholine (16.7 g.). The product was obtained as a colourless oil (10.2 g., 69%), b.p. 110-114°/0.1mm., $n_D^{17.5}$ 1.4680.

Found: N, 13.45 ; equiv. (titn.) 108.6 .

$C_{12}H_{26}N_2O$ requires N, 13.1% ; equiv. 107.2 .

Bis(6-piperidinohexyl)ethylamine.

The acid chloride in benzene (35 ml.), prepared from piperidinoadipamic acid (7 g.) as previously described, was added quite rapidly to a stirred solution of 6-piperidinohexylethylamine (13 g.) in benzene (50 ml.). After refluxing the solution for 45 minutes the precipitated 6-piperidinohexylethylamine hydrochloride was filtered off, and the filtrate evaporated to give a viscous dark oil. This was dissolved in chloroform (200 ml.), washed with water (2 x 20 ml.) and dried over sodium sulphate. 6-Piperidinohexylethylamine, (4.2 g., b.p. 110-117°/0.04 mm., $n_D^{16.5}$ 1.4700), was recovered from an aqueous solution of the hydrochloride by basifying, and extracting with chloroform (3 x 30 ml.).

Evaporation of the main chloroform solution yielded crude piperidinohexyl - N - ethyladipamoylpiperidine (12.5 g.) which was further dried in a vacuum desiccator before reducing with lithium aluminium hydride (3.5 g.) in

ether as described under 6-piperidinohexylethylamine. Fractional distillation gave a fore-run of the required product and 6-hydroxyhexyl-1'-piperidine, (1 g.), b.p. 129-222°/0.05 mm., and then the required product only (8.5 g., 68%), b.p. 226-232°/0.05 mm.

Found: N, 11.0 ; equiv. (titn.) 127.1.

$C_{24}H_{49}N_3$ requires N, 11.1% ; equiv. 126.6.

Bis(6-morpholinohexyl)ethylamine was prepared from morpholinoadipamic acid (5.5 g.) and 6-morpholinohexylethylamine (10 g.) by the method described for the synthesis of bis(6-piperidinohexyl)ethylamine. Fractional distillation gave a fore-run, (1.1 g.), b.p. 142 - 176°/0.03 mm., and a main fraction (6.2 g.) of pale yellow oil, b.p. 223 - 226°/0.03 mm.

Found: N, 11.1 ; equiv. (titn.) 127.2.

$C_{22}H_{45}N_3O_2$ requires N, 10.95% ; equiv. 127.9.

The fore-run was identical (equiv., infra-red spectrum) to the required product. Thus the total yield of the triamine was 74%. 6-Morpholinohexylethylamine (2.3 g., b.p. 111 - 112°/0.1 mm.) was recovered from the hydrochloride precipitated during the condensation reaction.

Quaternisation of bis(6-piperidinohexyl)ethylamine.

7-Ethyl-7-methyl-7-azoniatridecylenebis(N-methylpiperidinium) tri-iodide.

A solution of the base (0.67 g.) and methyl iodide (0.9 ml.) in ethanol (3.5 ml.) deposited a heavy, white crystalline precipitate on standing overnight. This was filtered off, washed with petrol (b.p. 40 - 60°) and recrystallised (methanol-petroleum ether, b.p. 40 - 60°) to give the required product, (1.3 g., 91%), m.p. 199 - 211° (shrivels and melts).

Found: N, 5.0 ; I, 47.2.

$C_{27} H_{58} I_3 N_3$ requires N, 5.2 ; I, 47.3%.

7, 7-Diethyl-7-azoniatridecylenebis(N-ethylpiperidinium) tri-iodide.

A solution of the base (0.88 g.) and ethyl iodide (1.8 ml.) allowed to stand overnight gave a sticky yellow oil which solidifed on drying in a vacuum desiccator. Recrystallisation (ethanol - ether) gave the product as a white microcrystalline powder which was collected by centrifuging, and similarly washed with ether (3 x 20 ml.) Yield 1.5 g. (76%), m.p. 226° (decomp.) with darkening at 214°.

Found : N, 4.9 ; I, 44.55.

$C_{30} H_{64} I_3 N_3$ requires N, 5.0 ; I, 44.9%.

Quaternary trichloroacetate

Trichloroacetic acid (1 g.) in water (1 ml.) added dropwise to a vigorously stirred solution of the semi-solid ethiodide (0.12 g.) in water (1 ml.) precipitated a yellow oil. The supernatant liquid was decanted and the oil triturated with a dilute solution of trichloroacetic acid (5%) giving a fine white powder which was filtered and dried *in vacuo*. The trichloroacetate was slightly hygroscopic and was not recrystallised.

Found: N, 2.9 ; equiv. (titn.) 488.9.

$C_{42}H_{67}Cl_{18}N_3O_{12}$ requires N, 2.9% ; equiv. 481.

Attempted preparation of 7-ethyl-7-n-propyl-7-azoniatridecylenebis(N-n-propylpiperidinium)tri-iodide.

A solution of the base (0.88 g.) and n-propyl iodide (1.8 ml.), allowed to stand for two days, gave a yellow semi-solid which yielded a fine, white powder on triturating with dry ether. This material was collected and washed by centrifuging with dry ether (3 x 20 ml.), but decomposed when dried *in vacuo*.

Quaternisation of bis(6-morpholinohexyl)ethylamine. 7-Ethyl-7-methyl-7-azoniatridecylenebis(N-methylmorpholinium) tri-iodide.

Methyl iodide (1.5 ml.) added to the base (1 g.) gave a sticky yellow solid which was triturated with dry ether and collected by centrifuging. The product was a very

hygroscopic yellow powder (1.2 g., 57%) and no melting point could be obtained.

Found: N, 5.1 ; I, 47.3.

$C_{25}H_{54}I_3N_3O_2$ requires N, 5.2 ; I, 47.0%.

Quaternary trichloroacetate

A semi-solid sample of the methiodide (0.5 g.) treated with a solution of trichloroacetic acid gave the trichloroacetate as a yellow powder after prolonged trituration. The product was filtered off, washed with water and dried in vacuo.

Found: N, 3.0 ; equiv.(titn.) 459.1

$C_{37}H_{57}Cl_{18}N_3O_{14}$ requires N, 3.0%. equiv. 468.7.

7, 7-Diethyl-7-azoniatridecylenebis(N-ethylmorpholinium) tri-iodide.

The base (1 g.) treated with ethyl iodide (1.8 ml.) gave a brittle, yellow solid on standing for two days. Precipitation from methanol-ethanol-ether yielded a flocculent white powder which was collected by centrifuging as previously described, and dried in vacuo, (1.4 g., 63%), m.p. 197 - 200°.

Found: N, 4.7 ; I, 44.9

$C_{28}H_{60}I_3N_3O_2$ requires N, 4.9 ; I, 44.7%.

Preparation of tetrahydropapaverine.

Tetrahydropapaverine, (1,2,3,4 - tetrahydro - 6, 7-dimethoxy-1-[3,4 - dimethoxybenzyl]isoquinoline), was obtained from the hydriodide by the extraction method of Pyman[127] and from papaverine by Craig and Tarbell's method of catalytic hydrogenation.[128]

Catalytic hydrogenation of papaverine.

Papaverine (13.7 g.) in ethanol (750 ml.) was shaken with Raney nickel (1 to 2 g.) for 4 hr. at 170° under hydrogen at 2000 lb/in.^{2} The catalyst was removed by filtration and the filtrate evaporated, cooled and filtered until all the unreduced papaverine had been removed. Finally, all the solvent was evaporated leaving the product as a viscous brown oil (10.8 g., 78%) which would not solidify. The infra-red spectrum and the melting point of the hydrochloride, 216°, were identical to those of the material prepared from the hydriodide.

Ethyl 2-tetrahydropapaverinyladipamate .

Ethyl adipoyl chloride in benzene (25 ml.), prepared from ethyl hydrogen adipate (7.5 g.), was added to a stirred solution of tetrahydropapaverine (14.5 g.) and triethylamine (10 ml.) in benzene (75 ml.), and the mixture gently refluxed for forty-five minutes. The precipitated triethylamine hydrochloride was filtered off and the filtrate washed with water (20 ml.), sodium

carbonate (20 ml. 10%.) and dried (Na_2SO_4).
Evaporation of the solvent gave the product as a viscous yellow oil (18 g., 84%) which would not solidify on prolonged drying in vacuo.

Found: N, 2.9 $C_{28}H_{38}NO_7$ requires N, 2.8%.

2 - Tetrahydropapaverinyladipamic acid.

2 - Tetrahydropapaverinyladipamic acid was obtained as a viscous oil by the saponification of the ester (17.5 g.) as described for piperidinoadipamic acid. The product was contaminated with a little adipic acid which was removed in the usual manner. The successive addition and evaporation of several volumes of dry ether followed by drying over potassium hydroxide gave a flaky, yellow powder, (15.5 g., 94%) m.p. 38 - 42° (decomp.).

Found: N, 3.0 ; equiv.(titn.) 468.9
$C_{26}H_{34}NO_7$ requires N, 3.0% ; equiv. 472.6.

N - Ethyladipamoyltetrahydropapaverine.

The crude acid chloride, prepared by heating 2 - tetrahydropapaverinyladipamic acid (5 g.) with thionyl chloride (1.5 ml.) at 70 - 85° for 25 to 30 minutes, was dissolved in benzene (40 ml.) and added to a stirred solution of anhydrous ethylamine (4 ml.) in benzene (30 ml.) at room temperature. The solution was stirred for 2 hr., filtered, washed with water (10 ml.) and sodium carbonate

(10 ml., 20%) amd dried over sodium carbonate. Evaporation of the solvent under reduced pressure gave the diamide as an oil (4.75 g., 90%) which was solidifed by drying in vacuo over potassium hydroxide pellets for several hours.

Found: N, 5.2. $C_{28} H_{39} N_2 O_6$ requires N, 5.6%.

6, 2' - Tetrahydropapaverinylhexylethylamine.

6, 2' - Tetrahydropapaverinylhexylethylamine was obtained as a dark yellow oil (3 g., 71%) by the lithium aluminium hydride (1.5 g.) reduction of the diamide (4.5 g.) in ether-tetrahydrofuran (100 ml., 1:1). A paper chromatogram (Whatmans No. 1), ascending method, using butanol - acetic acid - water (4 : 1 : 5) gave an R_f value of 0.56 to 0.61. An aliquot portion (1.5 g.) of the product in ether - methanol (80 : 1) chromatogrammed on an alumina column (8 x 2 cm.) gave the pure diamine (1.3 g.) in 175 ml. of eluate. The R_f value was unchanged.

Found: N, 5.8 ; equiv.(titn.) 242.8.

$C_{28} H_{43} N_2 O_4$ requires N, 5.9% ; equiv. 236.8.

Reineckate, purified by precipitation from acetone - ethanol - water as a pink powder.

Found: N, 16.9. $C_{36} H_{59} Cr_2 N_{14} O_6 S_8$ requires N, 17.1%.

Bis(6, 2' - tetrahydropapaverinylhexyl)ethylamine.

The crude acid chloride in benzene (50 ml.), prepared from 2-tetrahydropapaverinyladipamic acid (6.5 g.), was added to a stirred solution of 6,2'-tetrahydropapaverinylhexylethylamine (5.5 g.) and triethylamine (2 ml.) in benzene (30 ml.), and refluxed for one hour. The mixture was allowed to stand overnight, the precipitated triethylamine hydrochloride filtered off and more benzene (100 ml.) added to the filtrate which was washed with water (10 ml.) and sodium carbonate (10 ml., 20%) and then dried (Na_2SO_4). Distillation of the solvent gave a hygroscopic powder (11 g.) which on reduction with lithium aluminium hydride (3 g.) in a mixture of ether and tetrahydrofuran (100 ml., 1 : 1) gave the crude triamine, as a viscous yellow oil (8 g., 76%). A paper chromatogram of this oil, employing the method described for the diamine, showed extensive tailing, and no central spot was discernible. An aliquot portion (6 g.) of the oil in ether - methanol (80 : 1) was chromatogrammed on an alumina column (15 x 2 cm.), and the eluate collected in five arbitrary fractions of 150, 150, 75, 75 and 75 ml. Fractions one and two yielded pure triamine as a pale yellow oil, (3 g. and 1.5 g. resp.). The paper chromatogram now showed a

dense central spot, R_f 0.7, and only slight tailing. Ultra-violet absorption showed λ max. (in ethanol) 284 mμ (ε 10,600).

Found: N, 4.8 ; equiv.(titn.) 298.9 .

$C_{54} H_{79} N_3 O_8$ requires N, 4.7% ; equiv. 300.7.

The third fraction from the column also gave a yellow oil, (1 g.) which deposited a yellow solid on standing, whose infra-red spectrum showed a hydroxyl band at 3,200 to 3,700 cm.$^{-1}$ The fourth and fifth fractions gave only a yellow solid (0.4 g.) whose infra-red spectrum was very similar to that of the triamine but had a strong hydroxyl band at 3,200 to 3,700 cm.$^{-1}$ The ultra-violet spectrum (in ethanol) had λ max. 284 mμ (ε 6,540) and chromatography on paper using the system described for the diamine gave R_f 0.85 to 0.89. The material was 6'-hydroxyhexyl-2'-tetrahydropapaverine.

Found: N, 3.1 ; equiv.(titn.) 435.

$C_{26} H_{38} N O_5$ requires N, 3.15% ; equiv. 444.6.

Quaternisation of bis(6,2'-tetrahydropapaverinylhexyl) ethylamine.

7-Ethyl-7-methyl-7-azoniatridecylenebis(N-methyltetrahydropapaverinium)tri-iodide.

A solution of the triamine (0.42 g.) in dry ether (3 ml.) was treated with methyl iodide (0.75 ml.) and allowed to stand overnight. The product was obtained as

a yellow powder (0.5 g., 81%), by centrifuging and washing the precipitate with dry ether (3 x 20 ml.) and drying in vacuo. The melting point of the methiodide was very indefinite. Gladych and Taylor (87) have also observed that the quaternary salts of other tetrohydropapaverine derivatives melt over a wide range.

Found: N, 2.95 ; I, 28.8.

$C_{57} H_{88} I_3 N_3 O_8$ requires N, 3.2 ; I, 28.7%.

Attempted preparation of the triethiodide.

A solution of the base (0.28 g.) and ethyl iodide (0.5 ml.) in ether (2 ml.) allowed to stand for two days deposited a brittle yellow solid which was collected and washed as described for the trimethiodide. However, the product failed to analyse satisfactorily, and on subsequent preparations twice decomposed to a brown oil in the desiccator.

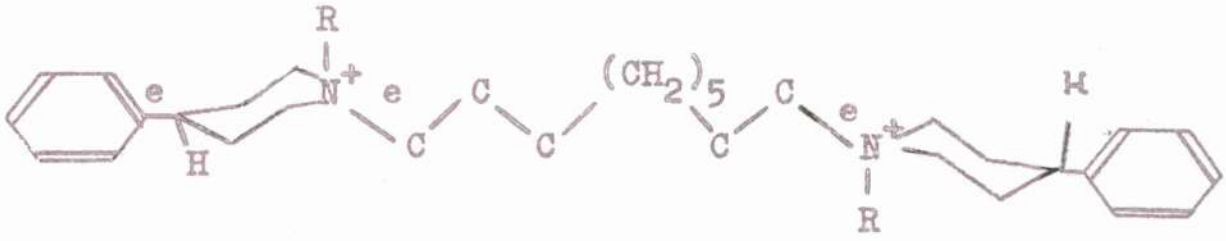
PART II

D I S C U S S I O N

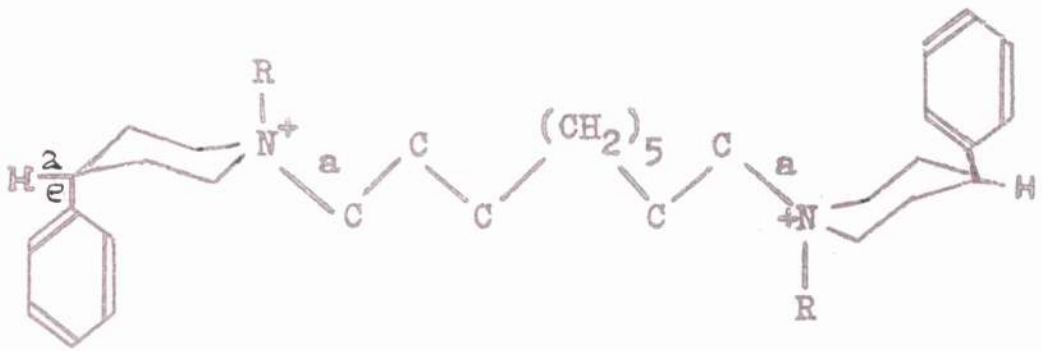
Introduction.

In continuation of the study of onium substituents it was thought desirable to examine some compounds having small steric variations of the onium head. According to Cavallito[35] steric factors could have a dual influence on the stability of the bond formed between a blocking agent and the receptor; besides influencing the ease and closeness of approach to the receptor the configuration of the substituents may also prevent the close approach of a displacing ion. This latter effect could influence the duration of action of the blocking agent.

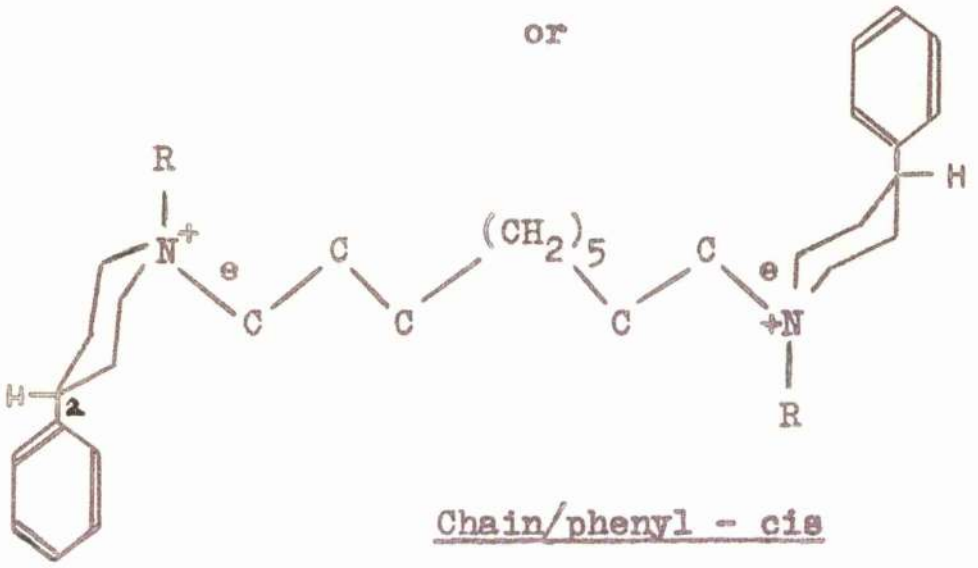
Initially, decamethylenebis(1-methyl-4-phenylpiperidinium)di-iodide was examined for the presence of geometric isomers resulting from cis and trans isomeriam, Figure 5.



Chain/phenyl - trans



or



Chain/phenyl - cis

(Fig. 5)

Beckett and Casey[134] and Standaert and Friess[25,i] have pointed out that the use of geometric isomers offers an approach to the elucidation of certain features of receptor structures and an opportunity to correlate differences in biological activity with steric and physicochemical properties. Although Mills, Parkin and Ward[135] were able to isolate isomeric mono-onium salts of 4-phenylpiperidine and so establish the tetrahedral configuration of the quaternary nitrogen atom, decamethylenebis(1-methyl-4-phenylpiperidinium)di-iodide appeared to exist in only one form, presumably trans which would be thermodynamically the more stable.

However, the starting material for the synthesis, 4-phenyl -1,2,3,6- tetrahydropyridine, presented the opportunity of examining some closely related compounds in which the conformations of the nitrogen - containing ring differed. 4-Phenyl-1,2,3,6-tetrahydropyridine could be readily dehydrogenated[136] to 4-phenylpyridine and the activity of three compounds, decamethylenebis(1-methyl-4-phenylpiperidinium)di-iodide, decamethylenebis(1-methyl-4-phenyl-1,2,3,6-tetrahydropyridinium)di-iodide and decamethylenebis(1-methyl-4-phenylpyridinium)di-bromide, compared.

The examination of models of 4-phenyl-1,2,3,6-tetrahydropyridine (LXV), 4-phenylpiperidine (LXVI) and 4-phenylpyridine (LXVII) reveals the conformations shown in Figure 6.

(LXV)

(LXVI)

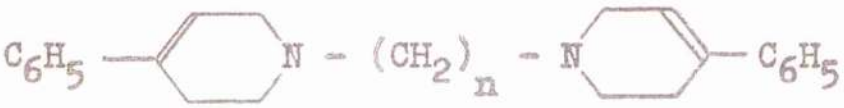
(LXVII)

(Fig.6)

Spectroscopic and dipole studies[137,138] have shown 4-phenylpyridine to be a planar molecule and that it retains its planar form in the methiodide.[137] Similarly it is unlikely that quaternisation would alter the half-chair (LXV) and chair (LXVI) conformations of the other two molecules. Thus a comparison of the type of action displayed by their bisonium compounds could probably provide further support for the postulates of Waser,[25,d] Nachmansohn[33,a] and Standaert and Friess[25,i] that the receptor has a pore-like structure. The flat 4-phenylpyridinium head will permit close approach to the receptor surface but it should be too large to fit into a receptor cavity, and should therefore have a (+)-tubocurare-like action; while the puckered nature of the other two compounds should permit some penetration, since the phenyl substituent in the 4-position provides relatively little steric hindrance to close approach. These compounds may therefore have a decamethonium-like action. However, differences in onium charge density among the three compounds cannot be entirely neglected and any difference in type or degree of activity between the two latter compounds may provide a more valid comparison for the elucidation of receptor features.

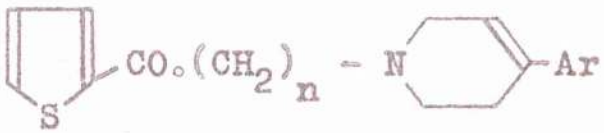
Although compounds containing the 4-phenyl-1,2,3,6-tetrahydropyridine system do not appear to have been

previously examined as neuromuscular blocking agents, many are of medicinal interest. Because of their structural relationship to pethidine these compounds have been generally investigated for analgesic properties. Bach and Vaughan[139] have prepared the inorganic acid salts of a number of compounds of general structure (LXVIII ; $\underline{n} \neq 10$)



(LXVIII)

which were useful analgesics, sedatives and hypotensive agents, but they did not prepare any quaternary salts. Janssen[140] has claimed anticonvulsant, hypnotic and analgesic properties for some 1-(2-thenoyl)alkyl-4-aryl-1,2,3,6-tetrahydropyridines (LXIX)

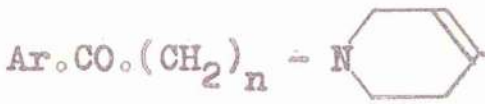


$(CH_2)_n$ = lower alkylene

Ar = substituted phenyl radical

(LXIX)

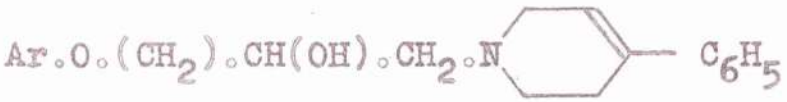
and for the corresponding aroyl compounds[141] (LXX).



Ar , Ar' = monocyclic aryl radicals.

(LXX)

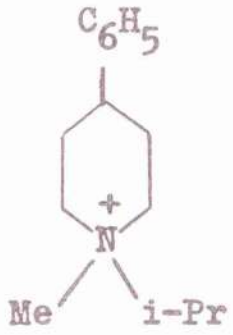
While the 4-phenyl-1,2,3,6-tetrahydropyridines (LXXI) prepared by Petrow, Stephenson and Thomas[142] are useful hypotensive agents.



Ar = substituted phenyl

(LXXI)

Several 4-phenylpiperidine derivatives have been reported to have ganglion or neuromuscular blocking properties. Chen and Parcell found[143] that 1,1-dimethyl-4-phenylpiperidinium iodide stimulated autonomic ganglia, but that 1-isopropyl-1-methyl-4-phenylpiperidinium iodide (LXXII) was a ganglion blocking agent. Mills, Parkin and Ward[135] have separated the geometric isomers of

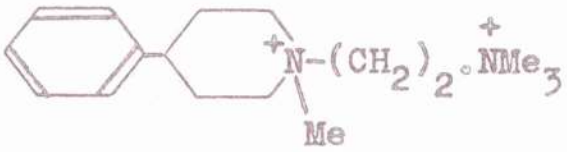


$I^{\ominus}$

(LXXII)

1-ethyl-1-methyl-4-phenylpiperidinium iodide but Chen and Parcell make no reference to the chemical homogeneity of compound (LXXII).

The asymmetric bisonium salts (LXXIII to LXXV) are also ganglion blockers of the autonomic and central nervous systems.(144)



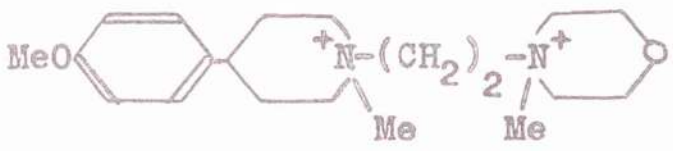
$2I^{\ominus}$

(LXXIII)

$2I^{\ominus}$

(n = 2,3)

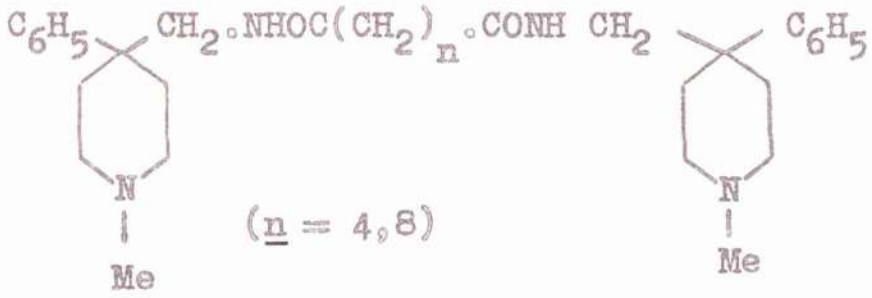
(LXXIV)



$2\ I^{\ominus}$

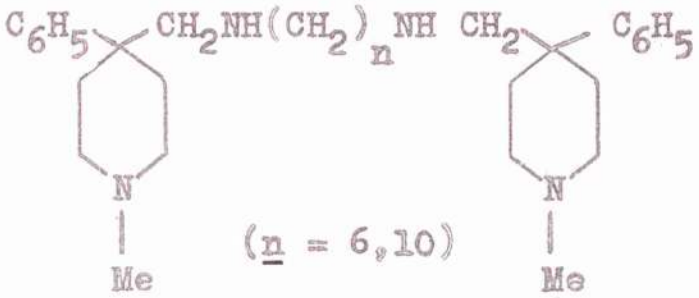
(LXXV)

Chiavarelli and Marini-Bettòlo and their colleagues have prepared compounds with curare-like,[145] strychnine-like[146] or hypotensive[147] actions by varying the nature of the second substituent in the 4-position of 4-phenylpiperidine. Of these the tertiary amines (LXXVI) are of particular interest since they provide one of the few examples of non-quaternary compounds with a curare-like action.[145]



(LXXVI)

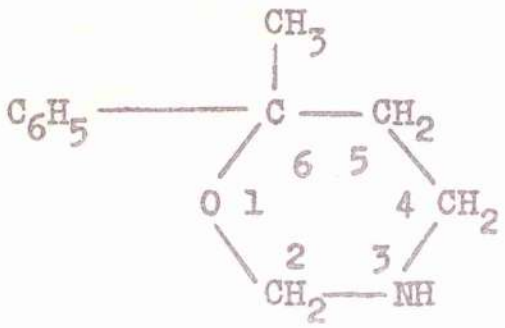
The di- and tetra-methiodides of the corresponding tetramines (LXXVII) also possessed neuromuscular blocking activity.



(LXXVII)

Discussion of experimental work .

Hartough, Dickert and Meisel[148] reported the preparation of 6-methyl-6-phenyltetrahydro-1,3-oxazine (LXXVIII) and various 3-alkyl-6-methyl-6-aryltetrahydro-1,3-oxazines



(LXXVIII)

from the condensation of α-methylstyrenes, formaldehyde and an ammonium salt. Schmidle and Mansfield[136] have shown that compound (LXXVIII) can be converted with excess acid to 4-phenyl-1,2,3,6-tetrahydropyridine (LXV), and

(LXV)

this method is of general application for the preparation of 4-aryl-1,2,3,6-tetrahydropyridines and 1-alkyl-4-aryl-1,2,3,6-tetrahydropyridines from the appropriate oxazines.[149,150,151,152] With the preparation of 4-phenyl-1,2,3,6-tetrahydropyridine the corresponding pyridine and piperidine derivatives became readily available. Dehydrogenation of compound (LXV) with nitrobenzene and a palladium catalyst has been reported [136] to give 4-phenyl pyridine (LXVII) in 81% yield, while catalytic hydrogenation of (LXV) over palladium yields 55% of 4-phenylpiperidine (LXVI). 1-Methyl-4-phenylpiperidine can also be prepared by this method,[151] but on dehydrogenation 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine undergoes N-demethylation to yield 4-phenylpyridine.[136]

The earlier preparations of 4-phenylpyridine either require starting materials which are not readily accessible or give low yields.[153]

4-Phenylpiperidine has previously been prepared from 4-phenylpyridine in good yield by reduction with sodium and ethanol[154] or by catalytic hydrogenation over a Raney nickel catalyst.[155] A direct synthesis involving the cyclisation of γ-cyano-β-phenylbutyric esters has also been employed.[156]

Although it has been reported that 1,2,3,6-tetrahydropyridines are difficult to reduce[157] and Schmidle and Mansfield hydrogenated 4-phenyl-1,2,3,6-tetrahydropyridine under forcing conditions, in the course of the present work it was found that 4-phenylpiperidine could be obtained fairly readily from 4-phenyl-1,2,3,6-tetrahydropyridine by sodium and ethanol reduction. This method was both rapid and simple and the yield (47%) comparable with that obtained by hydrogenation (55%).[136] Since tetrahydropyridines boil at about the same temperature as the corresponding piperidines[157] unreduced starting material could only be partially removed by fractional distillation and the product was purified by recrystallisation from n-heptane.

1-Methyl-4-phenylpiperidine was prepared in poor yield by the alkylation of 4-phenylpiperidine with methyl iodide by the method of Mills, Parkin and Ward[135], the reaction giving predominantly the quaternary methiodide. Reductive alkylation of 4-phenylpiperidine[156] also gives poor yields (44%). Therefore an attempt was made to obtain improved yields by the sodium borohydride reduction of 4-phenylpyridine methiodide, but this yielded 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine only.

Condensation of 4-phenyl-1,2,3,6-tetrahydropyridine with decamethylenedibromide in dry ethanol in the presence of sodium bicarbonate as described by Bach and Vaughan,[139] but modifying their isolation procedure, gave the required decamethylene diamine in 36% yield. No yield was reported by these workers. 4-Phenylpiperidine treated similarly gave a 29% yield of the diamine. However, the direct condensation of 4-phenylpiperidine with decamethylene-dibromide without the use of sodium bicarbonate gave a hydrobromide from which improved yields (43%) of the diamine were obtained.

The quaternary salts of both bases were prepared by adding excess methyl iodide to a refluxing solution of the base in chloroform. 4-Phenylpyridine and 1-methyl-4-phenylpiperidine were quaternised directly by refluxing with decamethylenedibromide in ethanol for several hours.

Decamethylenebis (4-phenylpiperidine) and decamethylenebis(4-phenyl-1,2,3,6-tetrahydropyridine) were quaternised in chloroform since Beasley, Petrow and Stephenson[158] found a sufficiently marked difference in solubility in a halogenated solvent to effect a separation of the two isomerides (LXXIX, R = Bz, R' = o - Me.C_6H_4. O.CH_2.CH OH.CH_2).

(LXXIX)

The methiodide of decamethylenebis (4-phenyl-1,2,3,6-tetrahydropyridine) was apparently completely insoluble in chloroform; however, evaporation of the chloroform filtrate from the quaternisation of decamethylenebis(4-phenylpiperidine) left a yellow powder (ca.1g.). From the recrystallisation of the main product three other fractions were obtained which appeared to differ in their solubilities in ethanol-water, the main fraction being the least soluble. The melting points of all four fractions differed, but their infra-red spectra were very similar. Descending paper chromatography, using as the solvent system, butanol-water-ethanol (4 : 1 : 5) gave only one compact spot in all cases. Fractions 2 to 4 were recrystallised and the melting points were then found to be similar to that of the main fraction. Since it appeared from the work of Mills, Parkin and Ward[135] that the ratio of the two isomers formed was dependent on the order of quaternisation

1-methyl-4-phenylpiperidine was quaternised with decamethylenedibromide, but the quaternary salt obtained by this method had an infra-red spectrum identical to those obtained previously. It was therefore assumed that only one isomer existed.

EXPERIMENTAL

Preparation of starting material.

4-Phenyl-1,2,3,6-tetrahydropyridine was prepared directly from α-methylstyrene, formaldehyde and ammonium chloride by the method of Schmidle and Mansfield.[136] The freshly distilled material was a colourless oil with a characteristic odour but turned a deep red colour after standing for several days and evolved a strong ammoniacal odour. Coloured material was redistilled before use, giving a product identical (infra-red spectrum, refractive index) to the freshly prepared material, b.p. 93-98°/0.6 mm., n_D^{23} 1.5900, λ max. 247 mμ (in ethanol) (ε 11,300). Lit.[136] gives b.p. 100 - 105°/1.5 mm., n_D^{25} 1.5882.

Found: N, 8.6. Calc. for $C_{11}H_{13}N$: 8.8%.

Hydrochloride, m.p. 202°, from acetone-isopropylalcohol. Lit.[136] gives m.p. 200 - 202°.

4-Phenylpyridine.

Oxidation of 4-phenyl-1,2,3,6-tetrahydropyridine with nitrobenzene and a palladium catalyst (5% Pd on charcoal) by the method of Schmidle and Mansfield[136] gave the required product (51%) as a white crystalline solid, b.p. 87 - 90°/0.5 mm. Recrystallisation from ethanol and n-heptane gave translucent platelets, m.p. 74°. Lit.[153,136] gives m.ps. varying from 73 to 78°.

The product purified by sublimation at 40°/0.1mm., melted at 74°.

Picrate, m.p. 194 - 197°. Lit.[136] gives m.p. 195 to 197°.

4-Phenylpiperidine.

A. Sodium (21.2g.) in finely sliced pieces was added to a refluxing solution of 4-phenylpyridine (10 g.) in dry ethanol (280 ml.) at a rate sufficient to keep the solution refluxing vigorously. When all the sodium had dissolved the mixture was allowed to cool for one hour during which time a gel formed. The sodium ethoxide was carefully neutralised with hydrochloric acid (100 ml.) and the ethanol removed under reduced pressure. The residue was made strongly alkaline with sodium hydroxide solution and extracted with ether. After drying (Na_2SO_4) evaporation of the solvent left a reddish oil which slowly crystallised. Fractional distillation gave 4-phenylpiperidine as a white crystalline solid (5.8 g., 56%), b.p. 82 - 86°/0.5 mm.).

Hydrochloride, white matted needles from ethanol-ether, m.p. 166 - 168°. Koelsch[156] gives m.p. 164 - 165°.

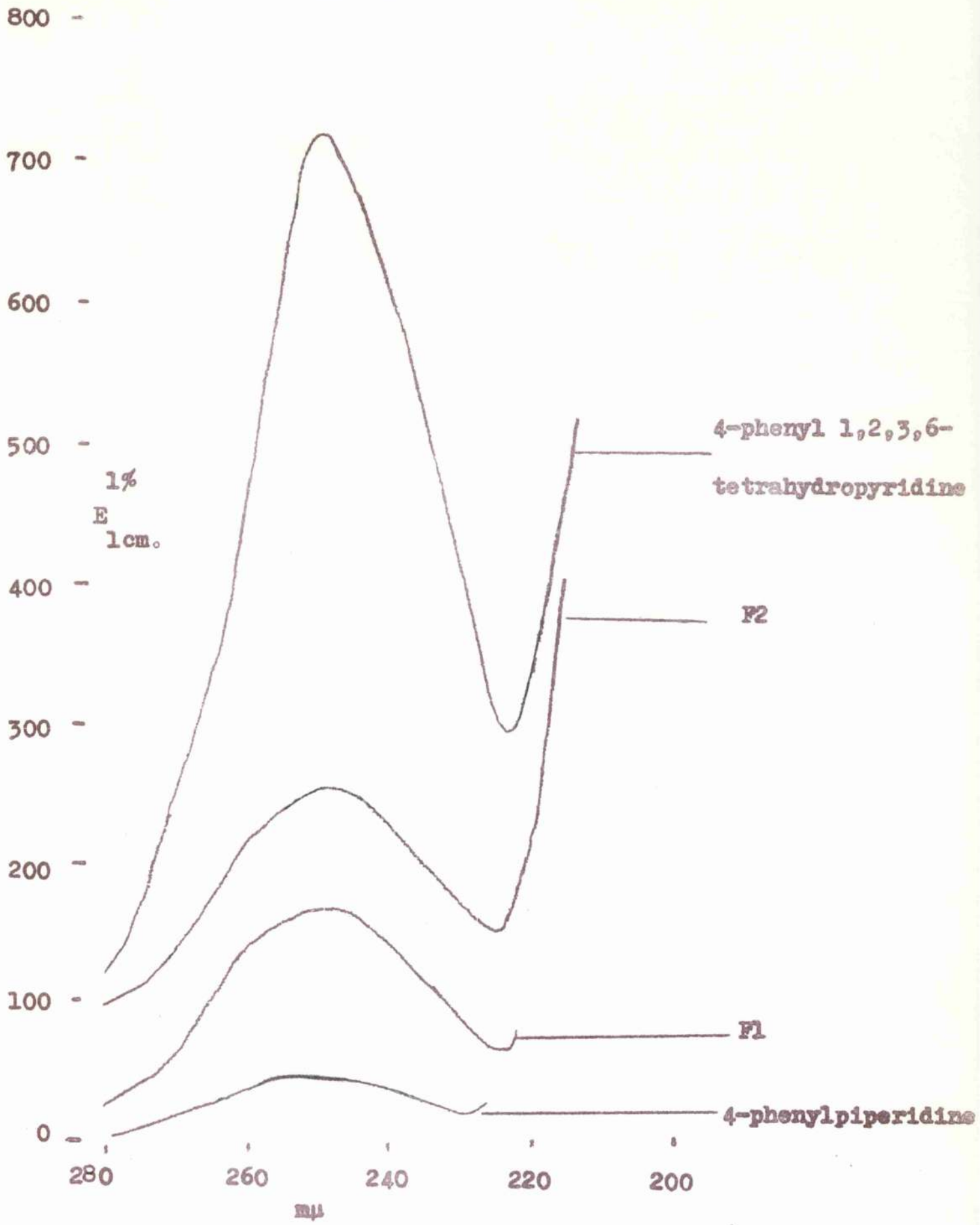
B. Sodium (23 g.) in small pieces was added over a period of 45 min. to a refluxing solution of 4-phenyl-1,2,3,6-tetrahydropyridine (23 g.) in dry ethanol (400 ml.). When all

the sodium had dissolved the solution was neutralised by the cautious addition of hydrochloric acid with external cooling. The ethanol was distilled off under reduced pressure, the semi-solid residue made strongly alkaline with sodium hydroxide (75 ml., 20%) and extracted with ether (500 ml.). After drying (Na_2SO_4) removal of the solvent gave a reddish oil (ca. 20 g.), λ max. 247 mμ (in ethanol)($E^{1\%}_{1cm.}$ 271). This material was fractionally distilled, b.p. 86 - 94°/1 mm., and two arbitrary fractions collected which gave the following $E^{1\%}_{1\ cm.}$ values :

	$E^{1\%}_{1\ cm.}$	λ max.
F 1	170	247 mμ
F 2	252	247 mμ

The $E^{1\%}_{1\ cm.}$ values of the starting material and the products are compared in the graph opposite. The two fractions were combined and recrystallised from n-heptane, collecting the crystals (11 g., 47%) under nitrogen. The product after two recrystallisations had m.p. 60 - 62°. Lit.(136,154,156) gives m.ps. ranging from 57 to 63°. Ultra-violet absorption (in ethanol) showed λ max. 251 mμ (ε 765).

Hydrochloride, m.p. 166 - 168°, from ethanol-ether. Lit.(156) gives m.p. 164 - 165°.


800
700
600
500
400
300
200
100
0
1%
E
1cm.
4-phenyl 1,2,3,6-
tetrahydropyridine
F2
F1
4-phenylpiperidine
280
260
240
220
200
mμ

1-Methyl-4-phenylpiperidine.

A. Employing the method of Mills, Parkin and Ward[135] 4-phenylpiperidine (8 g.) yielded 3.4 g.(39%) of product, b.p. 96°/25 mm. Hydrochloride, m.p. 199°, from methanol-ether. These workers give 1-methyl-4-phenylpiperidine b.p. 122 - 123°/11 mm., and hydrochloride m.p. 196 - 198°.

B. 4-Phenylpyridine (4.5 g.) in dry ether (40 ml.) was refluxed on the water bath with methyl iodide (6 ml.) for 15 min. and left overnight. The heavy, yellow precipitate was filtered off and washed with petroleum ether (b.p. 40 - 60°). Recrystallisation from ethanol-ether gave pale yellow, rhombic crystals (3 g.), m.p. 163 - 165° after drying in vacuo. A further yield of product (2 g.) was recovered from the mother liquor by evaporation and recrystallisation as above.

4-Phenylpyridine methiodide (no m.p. given) was previously prepared by Emmert and Varenkamp.[159]

Found: N, 4.6; I, 42.5.

Calc. for $C_{12}H_{12}IN$: N, 4.7; I, 42.7%.

Reduction of Methiodide.

The methiodide (4.5 g.) in methanol (90 ml.) and water (10 ml.) was refluxed with sodium borohydride (4.5 g.) for 15 min. on the water bath. More water (100 ml.) was added and the solution extracted with chloroform (150 ml.). Drying over sodium sulphate and evaporation of the solvent gave a pale yellow oil (2.3 g.). The addition of ether produced a light precipitate which was filtered off before distilling the oil, b.p. 97°/0.1 mm. The product was a very pale yellow, highly crystalline solid (1 g.), m.p. 37 - 39° after drying in vacuo. Lit.[151] quotes m.p. 40 - 42° for 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

The ultra-violet absorption (in ethanol) showed λ max. 247 mμ (ε 11,600). An ascending paper chromatogram (Whatman's No.1) using the system butanol-acetic acid-water (4 : 1 : 5) gave only one spot of R_f 0.75. Hydrochloride, acetone-ether, m.p. 252 - 254° with softening at 249°. Literature[151] gives m.p. 250 - 252° for the hydrochloride of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

Found: equiv.(titn.) 175.2. Calc. for $C_{12}H_{15}N$: equiv. 173.2. Methiodide, m.p. 266 - 270° (decomp.), from ethanol-ether.

Found: N, 4.4 ; I, 40.7

$C_{13}H_{18}IN$ requires N, 4.4 ; I, 40.3%

DECAMETHYLENEBISONIUM SALTS.

Decamethylenebis(4-phenylpyridinium)dibromide.

4-Phenylpyridine (3 g.) in ethanol (10 ml.) was heated with decamethylenedibromide (3 g.) at 85 - 90° on an oil bath for 14 hr. The reaction mixture was poured into petroleum ether (b.p. 40 - 60°) giving a heavy precipitate. The supernatant liquor was decanted and the precipitate recrystallised from ethanol-petroleum ether (b.p. 40 - 60°) to give the required product (1.5 g., 25%), as a microcrystalline white powder. The melting point after three crystallisations was 266 - 267° (decomp.).

Found: N, 4.5 ; Br, 26.3.

$C_{32}H_{38}Br_2N_2$ requires N, 4.6 ; Br, 26.2%

Decamethylenebis(4-phenylpiperidine).

A. Decamethylene dibromide (11.25 g.) and 4-phenylpiperidine (12.15 g.) in dry ethanol (135 ml.) were refluxed at 85° for 15 hr. in the presence of sodium bicarbonate (7.5 g.). The mixture was cooled and filtered, washing the residue with more ethanol before evaporating the filtrate to dryness. The product was treated with sodium hydroxide (100 ml., 20%), and extracted with chloroform (2 x 100 ml.). After drying (Na_2SO_4) removal of the solvent gave a red oil which crystallised fairly rapidly, m.p. 86 - 88°. The red colour could not be removed (charcoal) and distillation caused decomposition giving a mixture (8 g.), b.p. 95 - 220°/0.1 mm.

The residue originally filtered from the reaction mixture was insoluble in alkali, but was partially soluble in chloroform (100 ml.) leaving a residue of sodium bicarbonate. The chloroform extract was dried (Na_2SO_4) and evaporated to give a creamy solid, m.p. 95 - 97°. Recrystallisation from benzene or ethanol gave the required base (5 g., 29%), as white needles, m.p. 100 - 101° on drying in vacuo.
Found: C, 83.6 ; H, 9.8 ; N, 6.0 ; equiv.(titn.) 231.3.
$C_{32}H_{48}N_2$ requires C, 83.4 ; H, 10.5 ; N, 6.1%;
equiv. 230.4.

B. Decamethylene dibromide (17.5 g.) and 4-phenylpiperidine (19 g.) were refluxed in ethanol for $6\frac{1}{2}$ hr. during which a heavy precipitate of decamethylenebis(4-phenylpiperidine)dihydrobromide was formed. The reaction vessel was cooled and the product (27 g.) collected and dried in vacuo, m.p. > 250°.
Found: equiv. (non-aqueous titn.) 306.
$C_{32}H_{50}Br_2N_2$ requires equiv. 311.3.

The hydrobromide was shaken in a separating funnel with sodium hydroxide (30 ml., 50%) and chloroform (150 ml.) until it had almost all dissolved. The chloroform layer and a further extract (100 ml.) were dried (Na_2SO_4) and evaporated leaving a red oil which crystallised on cooling. The fine, white crystals from

ethanol-ether were washed with ether and dried *in vacuo*, m.p. 97 - 99°. Evaporation of the mother liquor gave another crop of crystals (2 g.), m.p. 97°. The total yield of the base was 11.5 g. (43%).
Found: N, 5.95%; equiv.(titn.) 233.3.

Decamethylenebis(4-phenyl-1,2,3,6-tetrahydropyridine).

A mixture of 4-phenyl-1,2,3,6-tetrahydropyridine (12 g.), decamethylene dibromide (11 g.) and sodium bicarbonate (7.5 g.) in ethanol (150 ml.) was refluxed for 15 hr. The warm reaction mixture was filtered and the sodium bicarbonate washed with a little chloroform. The required base which rapidly crystallised from the ethanol-chloroform filtrate on cooling was collected, and the filtrate chilled and refiltered. Most of the tertiary base was obtained by repeatedly concentrating the filtrate, chilling and filtering, until the precipitate gave a positive test for halide with silver nitrate. The solvent was then completely removed leaving a yellow solid (7.5 g.) which was basified with sodium hydroxide solution and extracted with chloroform to give a reddish gum which could not be further purified and was therefore discarded. The tertiary base recrystallised from ethanol had m.p. 116°. Bach and Vaughan[139] quote m.p. 114 - 116° . Total yield 6 g. (36%).

Decamethylenebis(1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)di-iodide.

Methyl iodide (2.7 ml.) added to a refluxing solution of the diamine (2 g.) in chloroform (10 ml.) gave a heavy, white precipitate of the product (2.1 g., 65%). Recrystallisation from ethanol-water gave a fine, white powder, m.p. 253 - 254° (decomp.) with darkening at 247°.

Found: C, 54.9 ; H, 6.6 ; N, 3.7.

$C_{34}H_{50}I_2N_2$ requires C, 55.1 ; H, 6.8 ; N, 3.8%.

Decamethylenebis(1-methyl-4-phenylpiperidinium)di-iodide.

Methyl iodide (20 ml.) was added to a solution of the diamine (15 g.) in chloroform (75 ml.) and the solution refluxed with stirring for 30 min. producing a heavy precipitate.

This was filtered, washed with chloroform and the combined filtrate and washings reserved. The precipitate was sparingly soluble in water and most organic solvents. Recrystallisation from ethanol-water (500 ml.) with chilling gave the product (14 g.) as a white microcrystalline powder, m.p. 259 - 260°. The filtrate was reduced to 100 ml. and chilled, yielding a further batch of white crystals. Evaporation of the filtrate left an oil which solidified on trituration with acetone-ether.

The filtrate reserved from the quaternisation reaction was evaporated giving an oil (1.5 g.) which was solidified by refluxing with acetone. The total yield of product from the four fractions was 20 g. (88%).

All four fractions were chromatographed on paper (Whatman's No.1). Their low solubility made it difficult to obtain a concentration which gave satisfactory spots, but this difficulty was overcome by using dimethylformamide. Each fraction gave only one spot of R_f 0.94. Recrystallisation of the three latter fractions from water-ethanol-ether gave m.ps. ranging from 255 - 260° (decomp.) after drying in vacuo.

Found: C, 54.85 ; H, 6.4 ; N, 3.75.

$C_{34} H_{54} I_2 N_2$ requires C, 54.8 ; H, 7.3 ; N, 3.8%.

Reineckate, decomp. p. 132 - 138°, recrystallised from acetone-water.

Found: Cr, 8.7 ; N, 17.15.

$C_{42} H_{70} Cr_2 N_{14} O_2 S_8$ requires Cr, 8.9 ; N, 16.85%.

Decamethylenebis(1-methyl-4-phenylpiperidinium)dibromide.

1-Methyl-4-phenylpiperidine (1.3 g.) in ethanol (5 ml.) was refluxed with decamethylenedibromide on a water bath for 7 hr. Dry ether was added and the solution filtered under nitrogen and washed with acetone, leaving a very

hygroscopic solid. This was refluxed with dry acetone, filtered and recrystallised from ethanol to give the product (1g., 54%), m.p. 272 - 274°. The infra-red spectrum of the material was identical with that of the quaternary di-iodide.

Found: C, 62.4 ; H, 8.6 ; N, 4.3.

$C_{34}H_{54}Br_2N_2$ requires C, 62.8 ; H, 8.4 ; N, 4.3%.

PHARMACOLOGICAL RESULTS

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RESULTS.

The compounds were tested for potency on the following preparations by the methods described by Carey and her colleagues:(37)

(a),(b) Cat and hen sciatic nerve-gastrocnemius muscle preparations,

(c) Rabbit head drop,

(d) Mouse inclined screen test.

CPD. NO. XL	$R_1R_2R_3N^+.(CH_2)_6.N^+R_4R_5.(CH_2)_6.N^+R_6R_7R_8$	Cat	Hen	Rabbit	Mouse	Ther. Index
A	Me_3 Me_2 Me_3	5.6	20	5.5	5.6	2.5
B	Me_2Et Me Et Me_2Et	22	65	15.3	13.4	2.1
C	Bu_2Me Bu Me Bu_2Me	33	130	23	7.6	1.9
D	Bu_2Et Bu Et Bu_2Et	82	197	56	29	2.6
E	Bu_2Pr Bu Pr Bu_2Pr	33	146	19	6.4	3.0
F	Bu_3 Bu_2 Bu_3	33	161	20	5.3	2.7

$[Cyc.N.(CH_2)_6]_2.N$ Et R

Pr = n-propyl, Bu = n-butyl
Cyc. = quat.nitrogen-containing heterocyclic group

G	1-methylpiperidinium	Me	53.3	440	33.6	28.9	1.5
H	1-ethylpiperidinium	Et	112	511.7	59.7	57.3	2.6
I	1-methylmorpholinium	Me	8.8	21	-	7.0	2.4
J	1-ethylmorpholinium	Et	10.8	48.9	7.3	6.3	2.3
K	2-methyltetrahydro-papaverinium	Me	116	439	68.7	57.3	2.6
	dtc		100	100	100	100	100

The therapeutic index was obtained from the ratio $\frac{\text{Mouse L.D}_{50}}{\text{Mouse P.D}_{50}}$ where L.D_{50} is the dose producing 50% fatalities in a group of mice and P.D_{50} is the dose required to paralyse 50% of the group.

Cpd. XL	Ganglion block		Cat respiration	Duration of paralysis (min.)		
	Dose factor	Inhibition		Cat	Hen	Rabbit
A	3.5	0	6.6	21	31	19
B	5	6%	21	18	34	9
C	5	0	25	18	-	8
D	10	0	75	19	-	8
E	5	0	24	21	33	7
F	10	0	29	22	-	7
dtc	5	38%	100	-	-	-
G	4	5%	60.2	17	24	29 (to recovery)
H	5	0	100	17	24	-
I	3.5	30%	10.9	22	42	-
J	4	20%	11.6	18	47	19 (to recovery)
K	5.5	2%	103	21	46	19 (to death)
dtc	4.5	67%	100	18	24	6 (to death) 14 (to recovery)

The average duration of paralysis (to complete recovery or death) in the cat, hen and rabbit preparations is also indicated in the above table. Ganglion blocking activity was estimated as the percentage block of the contraction of the cat nictitating membrane to stimulation of the superior cervical nerve, using dose levels several times greater (dose factor) than that required to produce 50% inhibition of the cat gastrocnemius muscle. The dose causing complete inhibition of respiration in the cat as determined by the asphyxial fall in blood pressure is also given. (+)-Tubocurarine (dtc.) was used as control in all these tests.

All the compounds exhibited a (+)-tubocurarine-like action. They had an additive effect when used in conjunction with (+)-tubocurarine and were antagonised by tensilon. Ether also potentiated their blocking activities.

CONCLUSION.

The results confirm the earlier findings of Carey, Edwards, Lewis and Stenlake[37] in the dihexazonium and dihexasulphonium compounds (Table 7). While (+)-tubocurarine-like activity is favoured by larger alkyl substituents the order of activity is ethyl > n-butyl > n-propyl $\gg$ methyl. The marked increase in activity with the introduction of a single ethyl group is also seen in the heterocyclic series. Thus these results support the postulate of Carey and her colleagues[37] that the anionic

receptor site may be associated with ancillary non-ionic sites complementary in size and shape to the ethyl group. In the heterocyclic series the morpholinium compounds, as was expected, showed a very low activity, but the piperidinium and tetrahydro-papaverinium compounds showed a fairly high level of activity comparable with the most active of the all alkyl compounds.

Although these trisonium compounds have a competitive action which appears to be even more specific than (+)-tubocurarine (less ganglion blocking activity), there are some interesting differences. They show a much greater species variation than (+)-tubocurarine and the order of activity, cat > rabbit > mouse, is the reverse of that proposed for (+)-tubocurarine by Paton.[44] The species variation and the very high activity in avian muscle are, in fact, features common to depolarising rather than competitive agents.

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