THE EFFECTS OF CHEMICAL STRUCTURE ON THE AFFINITY OF COMPOUNDS FOR ACETYLCHOLINE RECEPTORS

By

MD. GHULAM MUSTAFA
M.B., B.S., (DACCA).

Thesis presented for the Degree of
Doctor of Philosophy of the University
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INTRODUCTION

Mode of action of drugs:

It is usually supposed that drugs produce their biological effects as a result of physical or chemical interactions with cells. In many instances these interactions are limited to particular types of cell, e.g. muscle cells or nerve cells, and are a consequence of the combination of the drug with these cells. This combination may produce an effect comparable with normal physiological or biochemical processes, in which case the compound is referred to as an agonist. More often, the combination produces an effect by blocking normal processes and the compound is referred to as an antagonist.

Some substances, such as caffeine and normal aliphatic alcohols only produce their effects when given in amounts sufficient to form a monomolecular layer over the whole area of the cell (Clark, 1937a). This suggests that these drugs probably act by some physical or physicochemical process. Such a mechanism has been suggested, for instance, for the actions of anaesthetics (Meyer, 1901; Overton, 1901).

In contrast, Clark (1933) calculated that potent drugs such as acetylcholine, adrenaline, histamine and atropine could produce appreciable effects when given in amounts which could only cover a small fraction of the cell surface. These observations favour the idea, originally put forward by Langley in 1878, that many drugs act by combining with a small area on the cell

referred to as the "receptive substance" (Langley, 1905). This idea of "receptive substance" or "receptors" was used extensively by Ehrlich (1913) in his work in chemotherapy.

Similar ideas have been developed for the interactions of a substrate and the "active spots" on an enzyme (Michaelis and Menten, 1913) and for the adsorption of gas molecules on metal surfaces (Langmuir, 1916, 1918).

Clark (1937a) applied Langmuir's adsorption isotherm to the combination of drug with the receptor. If the drug, A, is combining reversibly with the receptors, R, giving a complex which somehow leads to a response,

$$A + R \rightleftharpoons AR \cdots\cdots\cdots\longrightarrow \text{response},$$

and if a proportion, y, of receptors is occupied by drug and the concentration of drug is A,

the rate of formation of the complex = $k_1 A(1-y)$

and the rate of break down of the complex = $k_2 y$.

At equilibrium, $k_1 A (1-y) = k_2 y$ and hence

$$KA = \frac{y}{1 - y} \qquad (I)$$

$$\text{or} \quad y = \frac{KA}{1 + KA} \qquad (II)$$

where $K = \frac{k_1}{k_2}$, the affinity constant.

If $A_{\frac{1}{2}}$ is the concentration which occupies half the receptors, $A_{\frac{1}{2}} = \frac{1}{K}$.

If the response is directly proportional to y then

when half the receptors are occupied the response will be half the maximum and by measuring the concentration of A which produces half the maximum response, we can obtain K. Although Clark suggested that affinity constants might be obtained in this way he himself said that the underlying assumptions were unlikely and these have subsequently been strongly criticised by Stephenson (1956).

If the ability of a drug to produce an effect depends upon the amount of complex formed, the affinity constant for the receptors will definitely be important because it determines the amount of the complex formed. This cannot be the only property involved, however, because some compounds, when adsorbed, do not produce an effect and act as antagonists, because they lack the ability to activate the receptors. In addition to antagonists there are compounds which have some ability to activate the receptors but which may not produce a maximum response from the tissue, however much is given. These have been called partial agonists (Stephenson, 1956) or competitive dualists (Ariëns, 1954) and it is assumed that these have an efficacy (Stephenson) or intrinsic activity (Ariëns) intermediate between potent agonists and antagonists. Paton (1961) has postulated that the ability of a drug to activate a receptor depends upon the rate of dissociation of the drug receptor complex, consequently partial agonists are compounds

with values of k_2 intermediate between those of agonists and antagonists.

Ariëns (1954) assumed that the response is directly proportional to the number of receptors occupied and to the intrinsic activity (α) of the agonist,

$$\text{Response} = \alpha y = \alpha \frac{AK_A}{1 + AK_A}$$

and K_A will be $\frac{1}{A_{50}}$, where A_{50} is the concentration producing a response which is 50 per cent. of the maximum of which the tissue is capable. Ariëns uses values of $\log \frac{1}{A_{50}}$ which he calls pD_2, as if it were a measure of the affinity and calculates α from the size of the maximum contractions which can be produced by the tissue.

Stephenson (1956), on the other hand, avoids this assumption by introducing another quantity, S, the biological stimulus, which is some function of R, the response, R = f (S). He defines the stimulus, S, as the product of efficacy (e_A) of the drug and the proportion of receptors occupied, i.e.,

$$S = e_A y$$

and hence

$$S = \frac{e_A AK_A}{1 + AK_A} \qquad \text{(III)}$$

so

$$\text{Response} = f(S) = f\left[\frac{e_A AK_A}{1 + AK_A}\right] \qquad \text{(IV)}$$

According to the theory of Paton,

$$\text{Response} = \phi k_2 y = \phi \frac{k_2 A}{A + k_2/k_1} \qquad \text{(V)}$$

Many compounds which act like acetylcholine appear to have the same intrinsic activity ($\alpha = 1$). This is very different from what is observed with the substrates of an enzyme where it is most unusual to find the substrates with the same value of k_3, the rate constant for the break down of enzyme-substrate complex into products. It raises the question whether the size of the maximum response is a function of the tissue rather than, or as well as, of the drug, i.e. whether some compounds do not stimulate the tissue so that it contracts as much as it is able, when y is only small (as suggested by Stephenson, 1956). Evidence for this has been produced by Nickerson (1956), who suggests that a maximum response of the tissue can be obtained by the combination of drugs such as histamine with only as little as 1 per cent. of the histamine receptors. Similar results have been reported by Furchgott (1955) with adrenaline and adrenergic receptors.

In these circumstances, when y is small,

$$AK_A = \frac{y}{1 - y} \longrightarrow y$$

i.e. response $= f(e_A y) = f(e_A AK_A)$

so, according to Paton's theory, $k_2 y = k_2 AK_A = k_1 A$

Even with this approximation, it is still impossible to obtain e_A or K_A. If two compounds, A_1 and A_2, produce identical responses, the biological stimulus produced will be the same for both, i.e. $e_1 A_1 K_1 =$

e_2 A_2 K_2, where the compound A_1, present in concentration A_1, has an efficacy e_1 and an affinity K_1 and the compound A_2, present in concentration A_2, has an efficacy e_2 and an affinity K_2. Although A_1/A_2 is known from the experiments there is still no means of obtaining separately e_1, e_2, k_1 and k_2.

The effects produced by an agonist, therefore, depend upon two parameters, its affinity (K) and its efficacy (e) or the dissociation-rate constant (k_2) and there is no simple means of estimating these separately.

As already mentioned, some drugs combine with receptors but do not activate them and block the actions of agonists. If A is the agonist and B the antagonist and both are present together,

$$A + R \rightleftharpoons AR \longrightarrow \text{Response}$$

i.e. Agonist + Receptor $\rightleftharpoons$ complex $\longrightarrow$ Response

$$B + R \rightleftharpoons BR$$

i.e. Antagonist + Receptor $\rightleftharpoons$ complex

If the agonist molecule in concentration (A) occupies a proportion, y, of the receptors and if the antagonist in concentration (B) occupies a proportion, z, we can write,

$$K_A = \frac{y}{(A)\ (1 - y - z)}$$

$$\text{or} \quad y = K_A(A)\ (1 - y - z) \qquad \text{(VI)}$$

where K_A is the affinity constant for agonist and the receptor.

$$K_B = \frac{z}{(B)\ (1 - y - z)}$$

$$\text{or} \qquad z = K_B(B)\ (1 - y - z) \qquad \text{(VII)}$$

where K_B is the affinity constant for the antagonist and the receptor.

From VI and VII we can derive,

$$(A)\ K_A = \frac{y}{(1 - y)}\ (1 + (B)\ K_B) \qquad \text{(VIII)}$$

When no antagonist is present this becomes $\frac{y}{(1 - y)}$ as equation (I).

Now, if the biological response to a concentration of (A) of agonist in the presence of a concentration (B) of antagonist is the same as that to a concentration (a) of agonist alone, it follows from equation (VIII),

$$\frac{A}{a} = 1 + (B)\ K_B \qquad \text{(Gaddum, 1937)}$$

$$\text{or} \qquad K_B = \frac{A/a - 1}{(B)} \qquad \text{(IX)}$$

A/a is called the dose-ratio; when A/a is 10, the concentration of the antagonist necessitates a 10-fold increase of agonist concentration in order to keep the response constant.

This equation is not based on any assumption about the relationship between biological stimulus and the size of the response, because the size of the response is kept constant. Moreover, the value of K_B will be the same whatever may be the concentration of antagonist used, so long as it acts competitively. This is an

absolute measure of the activity of an antagonist. In addition, it is independent of the affinity and efficacy of the agonist, provided only that the agonist and antagonist are competing for the same receptors.

Schild (1947) has applied this equation to devise a method for measuring antagonist activity. Responses are obtained with the agonist alone and then in the presence of a concentration, (B), of antagonist such that twice the concentration of agonist must be given to keep the response constant. The dose-ratio, therefore, is 2 and $\log \frac{1}{(B)} = \log K$.

Scott (Ph.D. thesis, 1962) has modified this procedure by testing a number of concentrations of antagonist and determined their dose-ratios. He used the values of the dose-ratios to obtain a number of estimates of the affinity constants and also plotted the (dose-ratio - 1) against (B), the antagonist concentration, to see whether the compound behaved competitively. Whatever method is selected, the activity of a competitive antagonist can be expressed in terms of its affinity.

Scott's method for measuring affinity constants was developed because he was interested in studying changes in affinity in series of antagonists obtained by replacing methyl substituents by ethyl in a trimethylammonium group. He studied pairs of series, e.g.

$RNMe_3^+$	$R'NMe_3^+$
$RNMe_2Et^+$	$R'NMe_2Et^+$
$RNMeEt_2^+$	$R'NMeEt_2^+$
$RNEt_3^+$	$R'NEt_3^+$

where the compounds in the first column were all antagonists and R was $Ph_2CHCOOCH_2CH_2$-, $Ph_2C(OH)COOCH_2CH_2$-, $Ph_2CHCH_2OCH_2CH_2$-, $Ph_2CHOCH_2CH_2CH_2$- and $Ph_2CHCH_2CH_2CH_2COCH_2$- .

He measured the affinity constants of all these compounds. The compounds in the second column were similar but lacked the two benzene rings, i.e. R' was $CH_3COOCH_2CH_2$-, $CH_3CH_2OCH_2CH_2$-, $CH_3OCH_2CH_2CH_2$- and $CH_3CH_2CH_2CH_2COCH_2$- and he measured their equipotent molar ratios relative to acetylcholine. Most of these compounds are agonists. It was thought that if the change in affinity, produced by replacing methyl by ethyl, was the same in the agonists as it was in the corresponding antagonists, it would be possible to assess the effect of replacing methyl groups by ethyl on the efficacy. When two agonists with affinity constants K_A and K'_A, and efficacies e_A and e'_A, respectively, produce comparable responses in concentrations A and A', the biological stimulus should be the same, i.e. if the proportion of receptors occupied is small,

$$e_A AK_A = e_{A'} A'K_{A'}$$

or $$\frac{A'}{A} = \frac{e_A K_A}{e_{A'} K_{A'}}$$

If $\frac{K_A}{K_{A'}}$ is known from the antagonists, it is possible to calculate $\frac{e_A}{e_{A'}}$

The suggestion that $\frac{K_A}{K_{A'}}$, for example, for $CH_3COOCH_2CH_2\overset{+}{N}Me_3$ and $CH_3COOCH_2CH_2\overset{+}{N}Me_2Et$, is the same as the ratio of the affinity constants of $Ph_2CHCOOCH_2CH_2\overset{+}{N}Me_3$ and $Ph_2CHCOOCH_2CH_2\overset{+}{N}Me_2Et$ is based on the following argument. The affinity constant, K, is related to the free energy of adsorption (ΔF) by the [illegible] equation,

$$\Delta F = -RT \log_e K_A$$

or $$\log_{10} K_A = \frac{-\Delta F}{2.3\ RT}$$

The change of methyl for ethyl increases the binding by an increment which could be due simply to the presence of the extra methylene group, consequently for the compound with one ethyl group,

$$\log_{10} K_{A'} = \frac{-(\Delta F + a)}{2.3\ RT}$$

and $$\frac{K_{A'}}{K_A} = \frac{-a}{2.3\ RT}$$

This is independent of ΔF and should, therefore, be the same for both agonists and antagonists, provided always that the onium group is bound in the same way in both the series of compounds, and that the replacement

of methyl by ethyl does not interfere with the binding of the rest of the molecule (i.e. alter ΔF in one series but not in the other).

In the five series of antagonists studied by Scott there was a fairly regular change in affinity with increasing replacement of methyl groups, even though the actual affinity constants differed by a factor of 200. The affinity was invariably increased by replacement of one or two methyl groups by ethyl but declined towards its original value when the third methyl group was replaced. The activity of those of the compounds which were agonists declined markedly with the replacement of methyl by ethyl and from this it was concluded that the change in structure was producing a marked change in efficacy.

The assumption that the effects of replacing methyl by ethyl are the same in the agonists as they are in antagonists has been criticised by Burgen (1965) who has obtained results which suggest that the onium group in the antagonists may be held further away from the negatively charged group on the receptors with which it interacts, than is the onium group in agonists. He suggested that the ability of the onium group to come close to the receptor may determine its ability to act as an agonist.

These ideas, however, are based only on observations with the two pairs of compounds, acetylcholine and

3-3-dimethylbutylacetate and benziloylcholine and (3-3-dimethylbutyl)benziloate and clearly much more information is needed. Results obtained by Abramson (1964) suggested that there were differences in the effects of chemical changes on the affinity of the series of antagonists when a bigger variety of groups was studied.

The aim of the present work was therefore:

I. To extend the work of Scott and Abramson to see whether the effects of changes in chemical structure on affinity are similar in various series of antagonists and, if they are not, to try to discern what similarity there is between the various series. Although Scott had found that the effects of replacing methyl by ethyl in the onium group were similar in five series of antagonists, Abramson found that replacement of methyl groups by pyrrolidino and piperidino groups had different effects in the diphenylacetyl and benziloyl derivatives, even though effects of replacing methyl by ethyl in these series were exactly the same.

II. To study the effect of temperature on the affinity of the compounds, and also to see if the affinity constants were the same when different agonists were used and also other tissues containing muscarine-sensitive acetylcholine receptors.

The compounds studied were:

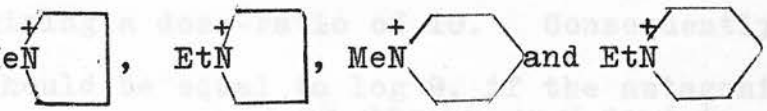
1. $Ph(CH_2)_5\overset{+}{N}R_3$, $PhCH_2COOCH_2CH_2\overset{+}{N}R_3$, Cyclo$Hex(CH_2)_5\overset{+}{N}R_3$

and <u>Cyclo</u>Hex$CH_2COOCH_2CH_2\overset{+}{N}R_3$.

2. $Ph_2CH(CH_2)_4\overset{+}{N}R_3$, $Ph_2CHCH_2OCH_2CH_2\overset{+}{N}R_3$ and <u>Cyclo</u>Hex(Ph)$CHCOOCH_2CH_2\overset{+}{N}R_3$.

3. Tropine and <u>pseudo</u>tropine meth- and eth-iodides and their benziloyl and diphenylacetyl esters.

In groups 1 and 2, $R_3\overset{+}{N}$ was $Me_3\overset{+}{N}$, $Me_2Et\overset{+}{N}$, $MeEt_2\overset{+}{N}$, $Et_3\overset{+}{N}$,



For convenience the compounds of group 1 are termed "lower analogues" of acetylcholine and those of group 2 "higher analogues" and those of group 3 "atropine analogues". The term "body" is used to describe the main bulk of the molecule apart from the onium group. In the series, in groups 1 and 2 the body is therefore the group $Ph(CH_2)_5$- or $Ph_2CH(CH_2)_4$-, etc., and in the compounds in group 3 it is the benziloyl or diphenylacetyl tropyl residue.

<u>Antagonists and competition</u>

The methods used for measuring affinity constants all assume that the antagonism is competitive. If it is not, the antagonism cannot be expressed in terms of an equilibrium constant. Although experiments may give what should be an affinity constant, this will not, in fact, be constant. For the work described in this

thesis it is most important to establish that the compounds are all competitive antagonists, acting in the same way as each other.

Schild (1947, 1957) and Marshall (1955) have used the difference between pA_2 and pA_{10} as a test for competition: pA_2 is the log of the reciprocal of the concentration of antagonist giving a dose-ratio of 2, and pA_{10} the corresponding value for the concentration giving a dose-ratio of 10. Consequently $pA_2 - pA_{10}$ should be equal to log 9, if the antagonism is competitive and the equilibrium is reached. This is not a particularly satisfactory test because of the size of the errors in the estimation of pA_2 and pA_{10}. Scott (1962) tested a number of concentrations of antagonist and plotted (dose-ratio - 1) against the concentration of antagonist. This should give a straight line passing through the origin and Scott found this to be so. In these experiments Scott obtained a log-dose-response curve and then exposed the preparation to the antagonist and increased the concentration of the agonist. From the responses to this concentration of agonist he calculated the dose-ratio and he then repeated the procedure with a higher concentration of antagonist. The disadvantage of this method is that, because responses are only obtained with one concentration of the agonist in the presence of a particular concentration of antagonist, there is no indication whether the antagonist has altered the slope of the log-

dose-response curve. An alternative procedure is to test many concentrations of agonist and observe whether the log-dose-response curve is parallel with original, obtained in the absence of antagonist (Gaddum, 1957; Schild, 1957). The disadvantage with this procedure, however, is that for a limited number of Responses only a limited range of concentrations of antagonist can be tested. Unless a wide range is tested, results may be obtained similar to those of Nickerson (1956) and Furchgott (1955) who found that some antagonists appeared initially to be competitive but were clearly non-competitive in higher concentrations. The apparently competitive phase could be explained by supposing that the action of the antagonist is really non-competitive, but that the agonist is occupying only a small proportion of the receptors. With the wide range of concentrations used by Scott it would seem most unlikely that a non-competitive antagonist could be mistaken for a competitive one, but in the course of testing the compounds listed above it has been necessary to develop other tests for competition using lower concentrations of the antagonists.

EXPERIMENTAL

Experimental

Preparations

1. The isolated guinea-pig ileum:

This preparation was set up as described by Stephenson (1956).

A guinea-pig, which had been starved for 24 hours and which weighed about 200-300 g., was killed by a blow on the head and bled out. The abdomen was opened and about 15 cm. of the terminal ileum was carefully dissected out and placed in a dish containing Tyrode's solution at about 30°C. The lumen of the gut was washed through with warm Tyrode from a pipette, with not more than 2-4 cm. of hydrostatic pressure to cause peristaltic evacuation. The terminal 3-4 cm. containing Peyer's patch was discarded and the adjacent 3 cm. of the ileum was mounted in an organ bath containing Tyrode's solution at 37°C., through which air was blown. One end of the gut was attached by a thread to a frontal writing lever writing on a smoked drum. The magnification of the lever was about five and the load was 0.5 g.

2. The isolated longitudinal muscle strip from guinea-pig ileum (Rang, 1964; Paton and Rothschild, 1965):

A piece of guinea-pig ileum, 4-5 cm. long, was freed from its mesenteric attachments and slipped, oral end first, over a pipette having an external diameter of 0.5 cm., which was held at an angle of about 30° to the

horizontal by a clamp. A flap of the longitudinal muscle coat was freed by gently rubbing the upper end of the ileum with a wad of moist cotton wool, starting at the mesenteric border. The process of peeling was continued on either side of the mesenteric border till the whole coat was freed at the upper end. A cotton ligature was tied to the free end of the flap. The muscle strip was then gently pulled downwards while the remainder of the gut was pulled upwards. The longitudinal coat was thus freed along its entire length without being torn except at the mesenteric attachments. A length of about 3 cm. of the muscle was then mounted in an organ bath in a way similar to that described for the whole ileum.

3. The isolated taenia coli of guinea-pig (Bülbring, 1954):

A guinea-pig was killed and the abdomen was opened. The colon was exposed and the taenia muscle was identified. A ligature was passed under it and tied and the bundle of muscle was cut near this ligature; care was taken not to penetrate the lumen of the gut. The cut end was lifted up by the thread and the bundle was separated from the underlying tissue by blunt dissection for about 10 cm.; this was transferred to a dish containing Tyrode's solution. About 3 cm. of the taenia coli was then cut off and a thread tied at each end; it

was then mounted in the organ bath. The lever had a magnification of about 7 and the load was 1 g. The temperature of the bath was 37°C.

4. The rat colon preparation (Clark and Raventos, 1937; Regoli and Vane, 1964):

A rat, weighing about 300 g., was killed by a blow on the head and bled out. The abdomen was opened and the colon was identified by the transverse striations over the ascending colon. The whole of the colon was removed and the lumen was cleaned by flushing with warm Tyrode's solution. A piece of colon about 2.5 cm. long was then mounted in the organ bath as described for the guinea-pig ileum.

The ascending colon and the transverse colon were usually found to be loaded with hard faecal matter and when tested were found to be less sensitive to carbachol than the descending colon. Usually, therefore, a piece of descending colon was used but even this took a longer time to settle down than did a piece of guinea-pig ileum; it was also slower in its response to drugs.

5. The isolated rabbit auricle preparation (Burn, 1952):

A young rabbit, weighing about 1000 g., was stunned by a blow on the head and bled out. The chest was opened and the heart was dissected out and placed in a dish containing oxygenated Locke's solution at about 30°C. All the tissues were quickly trimmed away until only the

auricles remained. A thread was attached to the tip of each of the auricles; one of the threads was tied to a fixed pin in the organ bath and the other was tied to a strain gauge (Force-displacement transducer, Model FT.03, without spring - by Grass). The organ bath, which had a capacity of about 45 ml., contained Locke's solution, well aerated with a mixture of oxygen (95 per cent.) and carbon dioxide (5 per cent.). The temperature was 37°C. and contraction of the muscle was recorded with a Devices Model M4-62 pen recorder.

Methods

1. The antagonist activity of the compounds was estimated by determining their affinity constants for the post-ganglionic acetylcholine receptors in the guinea-pig ileum, at 37°C.

In a few experiments, the method of Barlow, Scott and Stephenson (1963) was followed exactly, but in most of them two concentrations of agonist were tested in the presence of the antagonist, using a modification of the procedure, instead of only one concentration. The dose-ratio was measured by a 4-point assay. Responses were obtained with two concentrations of carbachol chloride (usually 6-8 x 10^{-8} M and 1.2-1.6 x 10^{-7} M); when these were steady, the Tyrode's solution was replaced by the Tyrode's solution containing the antagonist and the responses were obtained with higher concentrations of carbachol (usually 6-8 x 10^{-7} M and 1.2-1.6 x 10^{-6} M, because the concentration of the antagonist was deliberately chosen so as to produce a dose-ratio of about 10). In some experiments, however, higher concentrations of antagonist were tested and the concentrations of the agonist were increased as necessary.

The apparatus used was similar to that described by Stephenson (1956). The drug solutions were made up to the desired concentrations in Tyrode's solution and placed in reservoirs above the bath. At the appropriate time this was allowed to flow into the bath by the

machine and subsequently washed out by upward displacement with fresh solutions. The drugs were in contact with the tissue for 30 sec. and the preparation was left for 60 sec. to recover. The drugs were, therefore, added once in every 90 sec. When steady responses were obtained with the high and low concentrations of the agonist (usually within an hour from the starting of the experiment) a 6-way tap, connecting the bath with glass coils, was turned and the tissue was exposed to the Tyrode's solution containing antagonist, and the two higher concentrations of the agonist also containing the same concentration of antagonist. The cycle was then continued until these responses were also steady and roughly comparable with those obtained initially with agonist alone (Figure I).

The procedure, therefore, resembled a 4-point assay in that responses were obtained with high and low concentrations of the standard and high and low of the unknown (agonist and antagonist) but differed from it in that it was not possible to arrange the order in which these were given (in a random fashion or according to a Latin square).

The volume of the bath was 3 ml. and that of the glass coils, connecting the bath with the reservoir, was 25 ml. and the volume of the fluid to wash the preparation was 12 ml. and in these conditions sufficient fluid could run through the bath to effect complete exchange

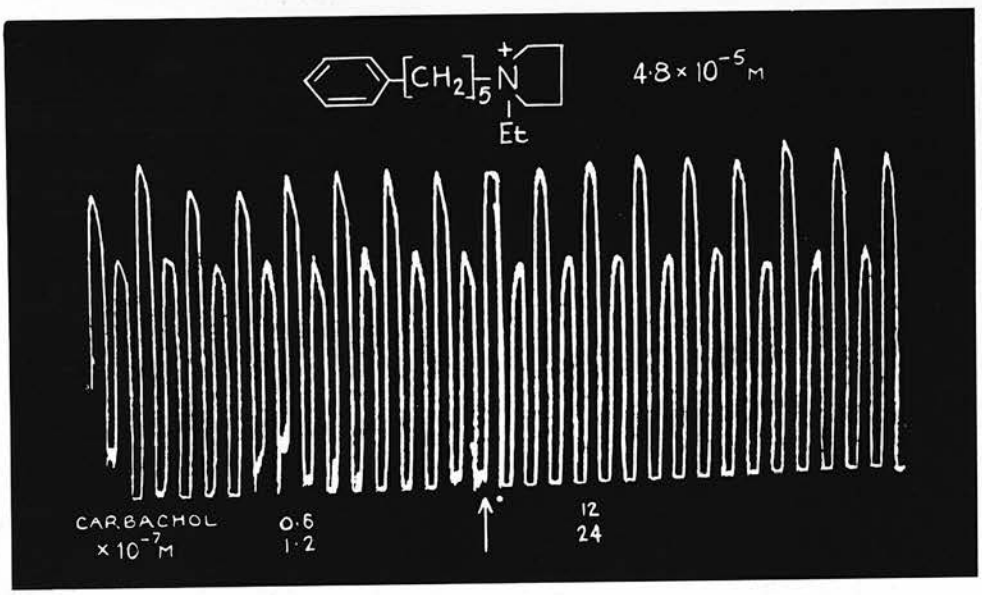


FIGURE I. Typical assay used to determine the affinity constant of an antagonist. Initial responses were due to carbachol, 0.6 and 1.2 x 10^{-7} M. At the arrow the Tyrode's solution was changed to Tyrode's solution containing the antagonist, phenylpentylethylpyrrolodinium 4.8 x 10^{-5} M, and the concentrations of carbachol were increased to 12 and 24 x 10^{-7} M.

without exposing the tissue to the air and without cooling more than $0.1^{o}C$.

Hexamethonium bromide, 2.75×10^{-4} M (100 mg./litre), was added to the Tyrode's solution to ensure that drugs were acting on post-ganglionic acetylcholine receptors.

All the experiments except those with rabbit auricles were performed with this procedure.

2. Rabbit auricles:

A few of the compounds were tested on the rabbit auricles in a similar way as on the guinea-pig ileum. The usual rate of beating was recorded and then a dose of carbachol was added to the bath from a blow-out pipette and rate of beating was again recorded. After 45 sec. the preparation was washed twice with Locke's solution and allowed to recover for 9¼ minutes (consequently a dose of carbachol was added once in every 10 minutes). The effect of the dose of carbachol was measured by calculating the percentage reduction in the rate of beating. When steady responses were obtained with high and low doses of carbachol the preparation was exposed to a concentration of the antagonist and responses were then obtained with still higher doses of the agonist (the concentration of the antagonist selected so as to produce a dose-ratio of 10). From the dose-ratio, the affinity constant was calculated as described above.

3. The effect of temperature on the affinity constant:

To see how the affinity constants of the antagonists vary with the temperature, a few of the compounds were tested both at 37°C. and 27°C. In some experiments the measurement was made first at 27°C. then at 37°C. and in other experiments the order was reversed.

The method of working out the results is based on the procedure described by Schild (1942) and by Gaddum (1954) and is illustrated by the following example:

Test compound, phenylpentyl (ethyl pyrrolidinium), 4.8×10^{-5} M (C)

Heights of contractions in mm.

	(i) Carbachol		(ii) Carbachol + (C)	
	6×10^{-8}	1.2×10^{-7}	1.2×10^{-6}	2.4×10^{-6}
	65	80	63	79
	65	78	62	80
	66	80	63	80
	65	80	62	78
	64	80	63	79
Total	325	399	313	396
Mean	65.0	79.8	62.6	79.2

From (i) and (ii),

The mean slope =

$$\frac{14.8 + 16.6}{2d} = \frac{31.4}{2d}$$

The preparation difference =

$$\frac{144.8 - 141.8}{2} = \frac{3.0}{2}$$

d = log. ratio between the two agonist doses

Hence, the log. dose-increment = $\frac{3.0 \times 0.301}{31.4} = 0.0288$

Hence taking antilog. and multiplying by 20, we get,

A/a (dose-ratio) = 1.069 x 20 = 21.38

A/a - 1 = 20.38, $K_B = \underline{4.25 \times 10^5}$

Each compound was tested on at least 5 different pieces of tissue, usually 7 or 8, and the mean of the logarithm of these individual values was calculated together with their standard error and the fiducial limits at a level of probability of 95 per cent.

4. A test for competitive antagonism:

In the present work the affinity constant of the antagonist was measured by applying Gaddum's equation (1937), i.e. assuming the compound to be a competitive antagonist. It was, therefore, necessary to check this. Barlow, Scott and Stephenson (1963) had tested several concentrations of the antagonist and found that the result fitted Gaddum's equation, i.e. the graph of dose-ratio minus one against the antagonist concentration was linear and passed through the origin (Fig.II)/. With less active

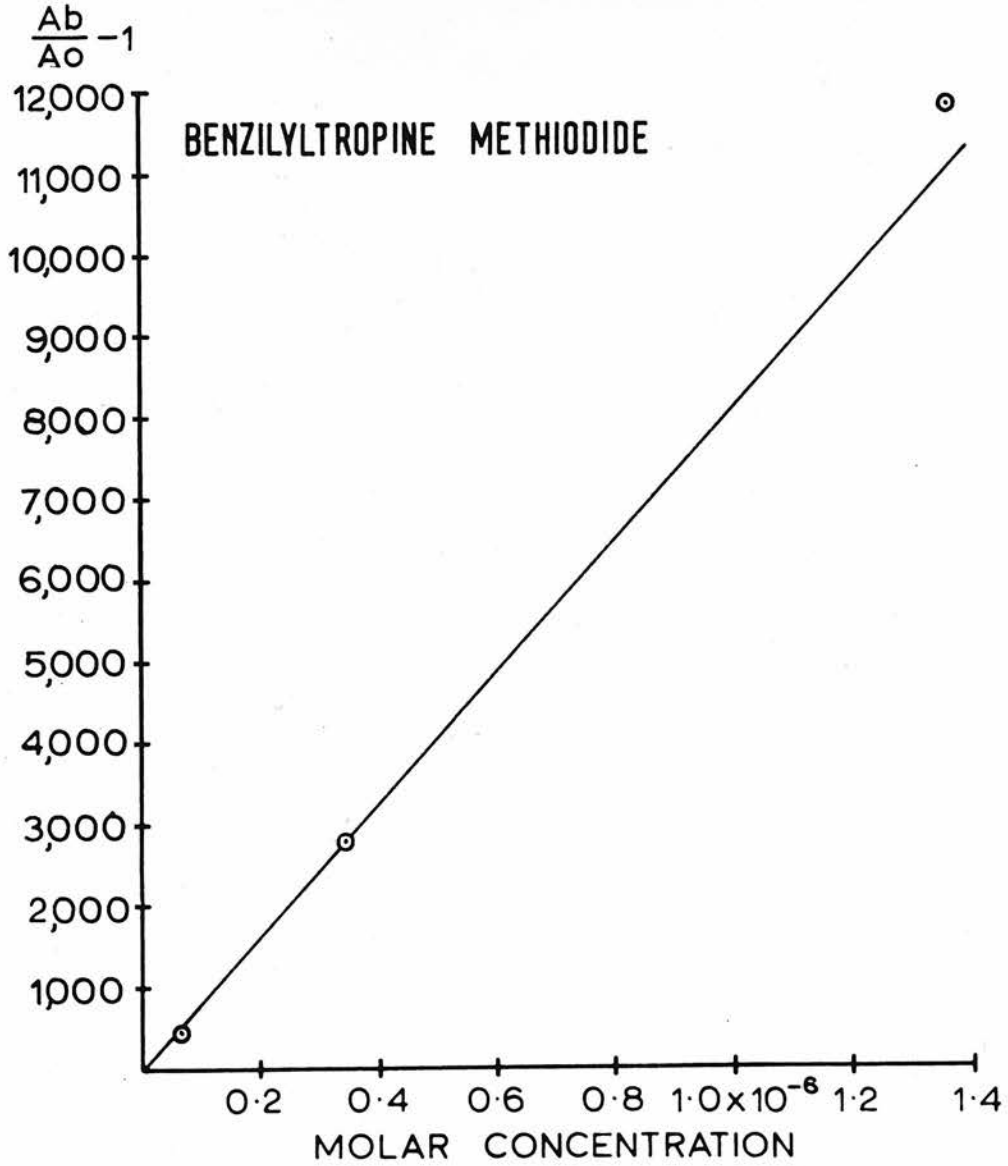


FIG.II. Graph of dose-ratio$(\frac{Ab}{Ao})-1$, against antagonist concentration. Linear relationship indicates competitive antagonism.

compounds it was not possible to test a wide range of concentrations. When some of the compounds were tested it seemed that they might not be acting competitively at high concentrations (Fig.IIA) so the following method was devised to see whether they were acting competitively in the lower concentrations.

Responses were obtained to the agonist and then to the agonist in the presence of a concentration (B) of atropine (a truly competitive antagonist) which produced a dose-ratio of about 100; the agonist was then tested in the presence of this concentration (B) of atropine together with a concentration of the antagonist under investigation (C) which by itself produced a dose-ratio of about 10.

If both the antagonists are competitive,

$$AK_A = \frac{y}{1 - y}(1 + BK_B + CK_C)$$

where y is the proportion of the receptors occupied by the agonist (whose concentration is A and affinity constant K_A) in the presence of a concentration B of the antagonist (atropine, affinity constant K_B) and a concentration C of the antagonist under test (affinity constant K_C). If the same responses were produced by a of the agonist alone, by A_b of the agonist in the presence of B, by A_c of the agonist in the presence of C and by A_{bc} of the agonist in the presence of both B and C together, it is reasonable to assume that y is the same

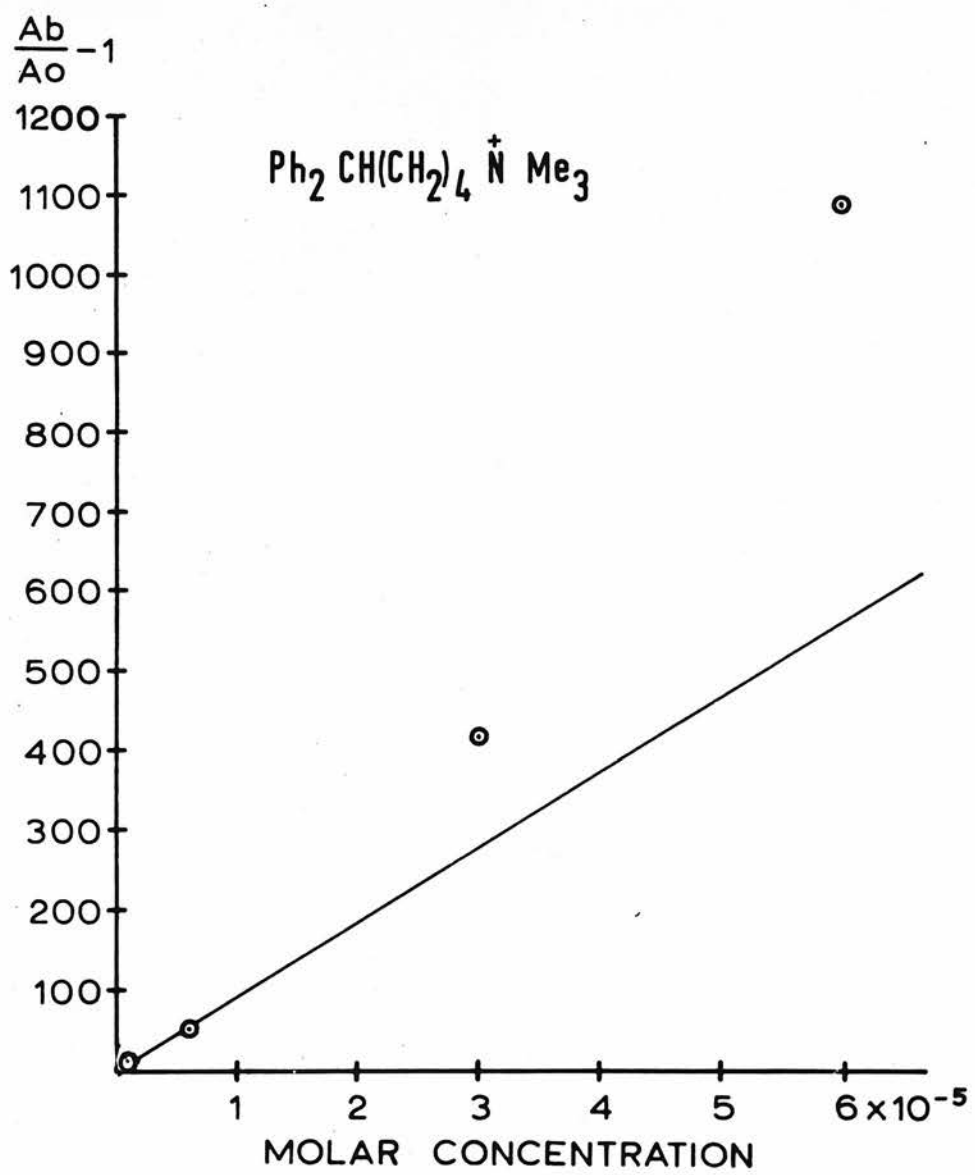


FIG. IIA. Graph of dose-ratio $(\frac{Ab}{Ao})-1$ against antagonist concentration. Linear relationship at lower concentration indicates competitive antagonism. Non-linear relationship at higher concentration indicates non-competitive antagonism.

in each situation and hence,

$$\frac{A_b}{a} = 1 + BK_B \qquad \text{(about 100 in this expt.)}$$

$$\frac{A_c}{a} = 1 + CK_C \qquad \text{(about 10 in this expt.)}$$

$$\frac{A_{bc}}{a} = 1 + BK_B + CK_C$$

and so, $$\frac{A_{bc}}{A_b} = 1 + \frac{CK_C}{1 + BK_B}$$

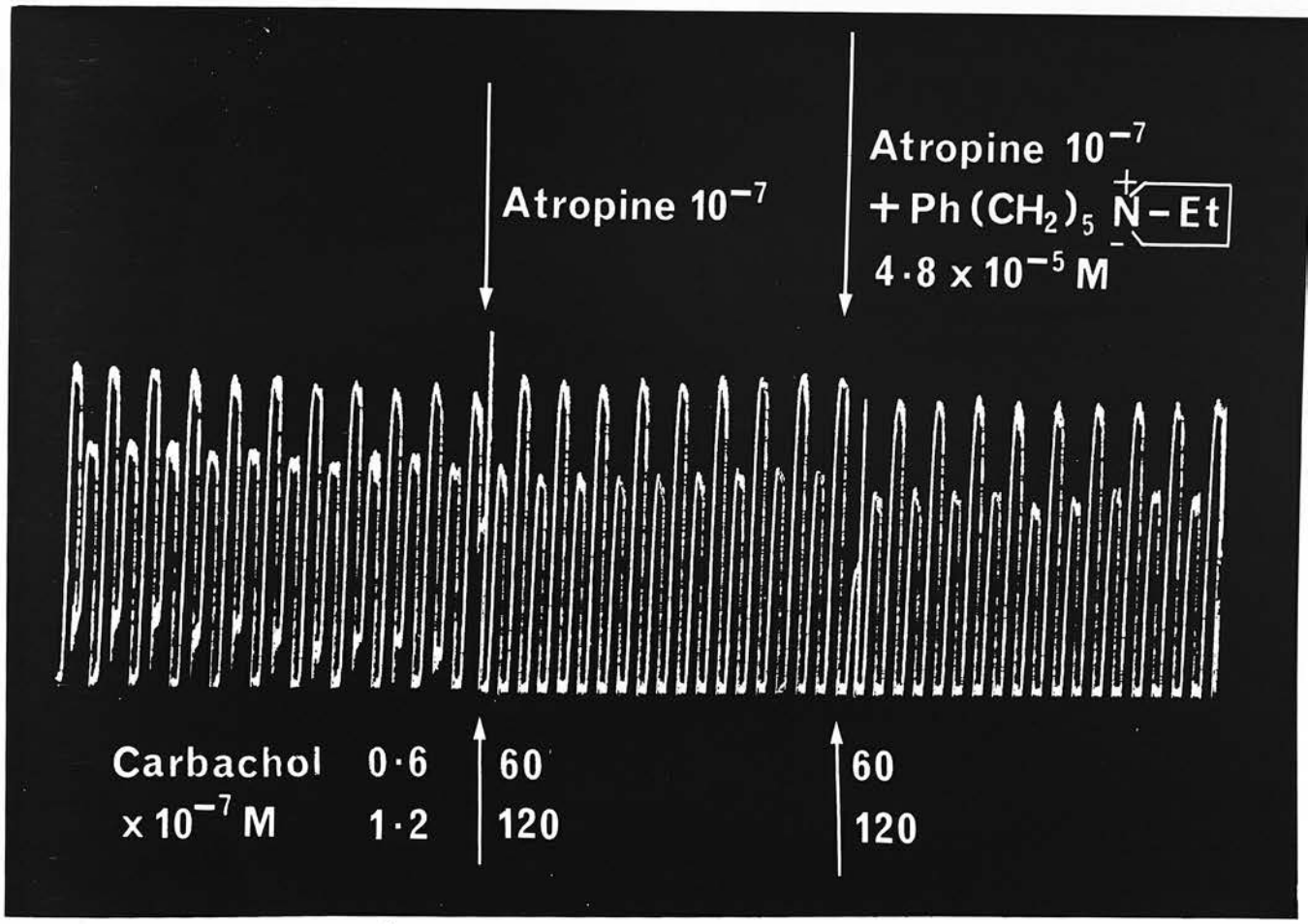
i.e., $$= 1 + \frac{\text{dose-ratio of C alone} - 1}{\text{dose-ratio of B alone}}$$

If the antagonist c is truly competitive the dose-ratio $\frac{A_{bc}}{A_b}$ should be slightly greater than one (about 1.09). But if C is not competitive and is not displaced from the receptors with increasing concentration of agonist, the dose-ratio $\frac{A_{bc}}{A_b}$ will be exactly the same as $\frac{A_c}{a}$, i.e. about 10.

Paton and Rang(1965-66) reported a similar test.

With this method it was easy to check that C was competitive at the lower concentration which was usually used for measuring affinity constants(Fig.III).

FIGURE III. The atropine test for competition. Initial responses were due to carbachol 0.6 and 1.2 x 10^{-7} M. At the first arrow the Tyrode's solution was replaced by Tyrode's solution containing atropine 10^{-7} M. The concentrations of carbachol were increased to 60 and 120 x 10^{-7} M and the drum was stopped for 15 minutes while preparation came into equilibrium with atropine and then restarted. At the second arrow the Tyrode's solution was again replaced by Tyrode's solution containing the test compound, Phenylpentylethylpyrrolidinium, 4.8 x 10^{-5} M and atropine 10^{-7} M and the drum was stopped again for 10 minutes, after which it was restarted. (Note that the concentrations of carbachol were not increased this time).


Atropine 10^{-7}
Atropine 10^{-7}
+ $Ph(CH_2)_5 \overset{+}{N}-Et$
$4 \cdot 8 \times 10^{-5}$ M
Carbachol
$\times 10^{-7}$ M
0·6
1·2
60
120
60
120

Test for competitive antagonism of carbachol with phenylpentyl (ethyl pyrrolidinium) 4.8×10^{-5} (C)

Example: Part 1 (Test compound (C) alone)

(1) Carbachol	Mean height (mm.)
6×10^{-8} M	75.4
1.2×10^{-7} M	85.8
(2) Carbachol with (C)	
1.2×10^{-6} M	75.4
2.4×10^{-6} M	86.8

From (1) and (2),

The mean slope $= \frac{10.4 + 11.4}{2d} = \frac{21.8}{2d}$

The preparation difference $= \frac{161.2 - 162.2}{2} = \frac{1.0}{2}$

Hence the log. dose-increment $= \frac{1.0 \times 0.301}{21.8} = \frac{0.0138}{.9862}$

Taking the antilog. and multiplying by 20 we get the dose-ratio $0.9687 \times 20 = 19.34$

Part 2 (Carbachol + atropine and carbachol + atropine + test compound (C))

(3) Carbachol	Height of contractions (mm.)
6×10^{-8} M	25.90
1.2×10^{-7} M	34.30
(4) Carbachol + atropine (10^{-7} M) (B)	
6×10^{-6} M	24.63
1.2×10^{-5} M	34.75
(5) Carbachol + atropine (10^{-7} M) (B) + test compound (C)	
6×10^{-6} M	23.00
1.2×10^{-5} M	33.00

From (3) and (4),

The mean slope $= \frac{8.40 + 10.12}{2d} = \frac{18.52}{2d}$

The preparation difference $= \frac{60.20 - 59.38}{2} = \frac{0.82}{2}$

Hence the log.dose-increment $= \frac{0.82 \times 0.301}{18.52} = 0.0133$

Taking the antilog. and multiplying by 100 we get,

$1.031 \times 100 = 103.10$ (dose-ratio for atropine 10^{-7} M (B) alone)

From (4) and (5),

The mean slope $= \frac{10.12 + 10.00}{2d} = \frac{20.12}{2d}$

The preparation difference $= \frac{59.38 - 56.00}{2} = \frac{3.38}{2}$

Hence the log. dose-increment $= \frac{3.38 \times 0.301}{20.12} = 0.0506$

Taking the antilog. and multiplying by 1 we get,

$1.120 \times 1 = \underline{1.120}$ (observed dose-ration for (B) and (C) together)

From Part 1 we had a dose-ratio for (C) alone of 19.34, so, from the formula, the theoretical dose-ratio for (B) and (C) together is,

$\frac{19.34 - 1}{103.10} + 1 = \underline{1.178}$ (observed value is 1.12)

RESULTS

Results

The estimates of the affinity constants (K_B) of the members of the 7 series of acetylcholine analogues are shown in Tables I - VII and summarised in Table VIIA. The estimates of the atropine analogues are shown in Tables VIII - XIII with a summary in Table XIV. The Table XV summarises the results of the tests with atropine for the competitive antagonism of some of the antagonists. The effect of temperature on affinity is shown in Table XVI. The effect on affinity for using different agonists is shown in Tables XVII - XIX. The variation of affinity constant with different tissues is shown in Tables XX - XXIII.

These tables show the individual estimates of affinity, the values of log K_B, the mean value of log K_B with its upper and lower limits at a level of probability of 0.05 shown in the parentheses.

The standard error of the mean, on the average, for the acetylcholine analogues was 5.5 per cent. The minimum percentage of error for these analogues was 1.8 per cent. and only in two compounds the percentages of error were higher than 10, having values of 11.5 and 11.9 per cent. Similar errors have been observed in the estimates of pA_2 and pA_{10} values (Timms, 1956) and both Scott (1962) and Abramson (1964) reported a standard error in affinity constant of 1 - 10 per cent. on the guinea-pig ileum.

Acetylcholine analogues:

1. The phenylpentyl series (Table I)

The affinity constants for the members of the series were calculated from experiments in which the dose-ratio was mostly about 10. In some experiments with triethyl-ammonium compound it was higher and even as high as about 300 but this did not appear to affect the result. With the ethylpyrrolidinium compound, however, there was an apparent increase in affinity constant with higher concentrations. Only the results with lower concentrations were included and at these concentrations the compound appeared to be competitive when tested by atropine method (page 26). The standard error of estimates for each member lies within 2-6 per cent.

The mean value of log K_B for the series lies between 5 and 6. The affinity increases by about two-fold for each successive replacement of methyl by ethyl up to triethyl-ammonium; this increase is statistically highly significant. With the introduction of a pyrrolidine ring the affinity fell sharply to a value less than that of the methyldiethyl-ammonium compound. The ethylpyrrolidinium and methylpiperidinium compounds have much the same affinity but that of ethylpiperidinium compound is again lower, comparable with that of the ethyldimethyl ammonium compound.

2. The cyclohexylpentyl series (Table II)

Experiments in which the dose-ratio was 10-20, were

TABLE I

PHENYL PENTYL SERIES

+ NR_3	Dose-ratio from each preparation	K_B	Log K_B	Mean log K_B (±S.E.) with 95% Fiducial Limits		Standard error as per cent. of mean	No. of Results
	10.0	1.57×10^5	5 .796				
+ N-Me_3 with Acetyl Choline	9.0	1.28	.107	5.189			5
	10.0	1.45	.161	(±0.024)			
	11.0	1.69	.228				
	10.0	1.79	.253		(5.141)		
					5.179	5	10
	8.4	1.48	.170		±0.017		
+ N-Me_3 with Carbachol	8.5	1.51	.179	5.170	(5.217)		
	7.3	1.26	.100	(±0.024)			5
	8.0	1.40	.146				
	10.0	1.79	.253				
	6.0	2.61×10^5	5 .417				
	9.4	3.00	.477	(5.379)			
+ N-$MeEt_2$	8.8	2.78	.444	5.446		4.3	5
	10.0	3.21	.507	(±0.021)			
	8.3	2.44	.387	(5.513)			
	10.0	4.70×10^5	5 .672				
	12.0	5.50	.740				
+ N-$MeEt_2$ with Acetyl Choline	11.6	5.78	.762				5
	11.0	4.93	.693	5.710			
	23.4	4.60	.663	±0.061	(5.683)	3.22	11
					5.714		
	15.5	5.78	.762	5.720	(±0.014)		
	13.5	5.00	.699	(±0.024)	(5.745)		6
with Carbachol	14.4	5.36	.729				
	16.0	6.05	.782				
	13.7	4.24	.627				
	17.0	5.31	.725				

TABLE I (Contd.)

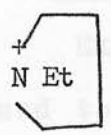
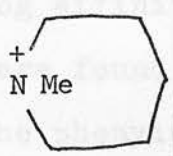
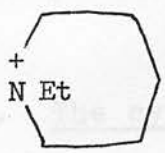
$+NR_3$	Dose-ratio from each preparation	K_B	Log K_B	Mean log K_B (±S.E.) with 95% Fiducial Limits	Standard error as per cent. of mean	No. of Results
	20.0	7.74×10^5	5 .889			
	17.0	8.20	.914			
	34.0	8.20	.914			
+ N - Et_3 with Acetyl Choline	19.0	7.12	.853	5.900		7
	18.0	8.50	.929	±0.010		
	26.0	8.42	.925	(5.875)		
	33.0	8.00	.903	5.894	2	13
	35.60	7.95	.900	±0.009		
	18.3	6.94	.841	5.880 (5.913)		6
with Carbachol	311.5	7.76	.890	(±0.014)		
	74.0	7.30	.863			
	23.0	8.80	.945			
	19.0	7.18	.850			
	17.8	4.20×10^5	5 .623			
	16.9	3.97	.599	(5.588)		
+ N Me	17.5	4.12	.615	5.632		6
	17.0	3.97	.599	(±0.017)	3.9	
	8.0	5.15	.712	(5.676)		
	9.8	4.40	.644			
	16.0	3.75×10^5	5.574			
	79.4	4.59	.662	(5.553)		
+ N Et	16.2	3.80	.580	5.631	6.4	5
	22.0	5.26	.721	(±0.028)		
	17.6	4.15	.618	(5.709)		
	21.3	5.07×10^5	5 .705			
	21.5	5.12	.709	(5.643)		
+ N Me	17.3	4.08	.611	5.684		6
	15.2	4.74	.676	(±0.016)	3.7	
	15.5	4.81	.682	(5.725)		
	16.8	5.26	.721			
	9.5	2.58×105	5 .412			
	10.0	3.00	.477	(5.412)		
+ N Et	10.0	2.98	.474	5.456	3.7	5
	10.4	3.13	.496	(±0.016)		
	9.0	2.63	.420	(5.500)		

TABLE II

CYCLOHEXYLPENTYL SERIES

$+NR_3$	Dose-ratio from each preparation	K_B	Log K_B	Mean log K_B ($\pm$S.E.) with 95% Fiducial Limits	Standard error as per cent. of mean	No. of Results
$+N\text{-}Me_3$	10.7	3.23×10^5	5 .509			
	8.9	2.63	.420	(5.339)		
	8.4	2.46	.391	5.411	6	5
	10.4	2.35	.371	($\pm$0.026)		
	10.3	2.32	.366	(5.483)		
$+N\,Me_2Et$	18.4	7.24×10^5	5 .860			
	21.4	8.50	.929			
	19.7	7.77	.890			
	9.7	5.44	.736	(5.761)		
	10.9	6.16	.790	5.825	6	7
	15.8	6.17	.790	($\pm$0.026)		
	10.6	5.97	.776	(5.889)		
$+N\,MeEt_2$	11.5	6.45×10^5	5 .816			
	15.6	9.11	.960			
	9.2	6.89	.838	(5.789)		
	10.2	7.69	.886	5.853 ($\pm$0.025)	5.8	6
	8.4	6.12	.787			
	9.1	6.76	.830	5.917		
$+N\,Et_3$	9.8	10.97×10^5	5 1.040			
	6.2	6.48	.812			
	14.5	11.25	1.051			
	12.4	9.49	.977	(5.835)		
	12.5	9.60	.982	5.922	8.5	8
	8.7	6.42	.802	($\pm$0.037)		
	10.5	7.95	.900	(6.009)		
	8.6	6.36	.804			

TABLE II (Contd.)

$^{+}NR_3$	Dose-ratio from each preparation	K_B	Log K_B	Mean log K_B ($\pm$S.E.) with 95% Fiducial Limits	Standard error as per cent. of mean	No. of Results
	8.5	7.47×10^5	5.873			
	8.2	7.18	.856			
	5.0	3.98	.600			
	7.4	4.24	.627	(5.584)		
	6.0	3.31	.520	5.702	11.5	8
	5.3	3.55	.550	$\pm$0.050		
	14.0	6.58	.818	(5.820)		
	12.7	5.87	.769			
	8.1	6.24×10^5	5.795			
	7.5	5.61	.749			
	9.3	7.24	.860	(5.768)		
	8.1	6.21	.793	5.814	4	6
	8.5	6.56	.817	($\pm$0.018)		
	9.5	7.38	.868	(5.860)		
	14.2	10.97×10^5	5 1.040			
	12.6	9.64	.984			
	8.4	6.14	.788	(5.855)		
	10.9	8.21	.914	5.919	6	8
	11.6	8.66	.947	($\pm$0.027)		
	11.0	8.38	.923	(5.983)		
	9.4	6.97	.843			
	10.7	8.12	.910			
	9.4	8.38×10^5	5 .923			
	9.4	8.45	.927	(5.917)		
	12.1	11.15	1.050	6.025	9.7	6
	10.2	9.16	.962	($\pm$0.042)		
	15.7	14.7	1.167	(6.133)		
	14.3	13.27	1.120			

used to calculate the affinity constants. The mean log affinity constant lies between 5.0 and 6.0 and the standard errors lie within 6-12 per cent. Though the affinity for the members of the series is a little higher than for the phenylpentyl series, the change of affinity with structure is similar in the two series up to the methylpyrrolidinium compound, after this the affinity of the cyclohexyl compounds continued to increase, unlike the phenylpentyl series in which it declined (Fig. IV, page 48).

3. The phenylacetoxy ethyl series (Table III)

Experiments in which the dose-ratio was 4-20, were used to calculate the affinity constant. The values obtained for the affinity constants were the lowest amongst the acetylcholine analogues tested and the means of the log affinity lie between 4.5 - 5.8. The standard errors were found to lie between 1.8 - 9.0 per cent. As with the phenylpentyl series the affinity sharply rises with the successive ethylation up to triethyl compound. With pyrrolidinium and piperidinium compounds the ethylated members had significantly higher affinity than their methylated homologues.

4. The cyclohexylacetoxyethyl series (TableIV)

Only the first four members of this series were tested. Experiments in which the dose-ratio was 7-26 were used to calculate the affinity constants. The

TABLE III

PHENYLACETOXYETHYL SERIES

$+NR_3$	Dose-ratio from each preparation	K_B	Log K_B	Mean log K_B ($\pm$S.E.) with 95% Fiducial Limits	Standard error as per cent. of mean	No. of Results
$+NMe_3$	4.0	3.73×10^4	4.572			
	9.0	3.29	.517			
	10.0	3.80	.580	(4.504)		
	9.2	3.43	.535	4.533		
	9.1	3.36	.526	($\pm$0.012)	2.8	7
	8.4	3.26	.513	(4.562)		
	8.0	3.07	.487			
$+NMe_2Et$	16.9	1.32×10^5	5 .121			
	20.7	1.64	.215			
	13.5	1.04	.017	(5.038)		
	15.9	1.24	.093	5.099		
	16.0	1.25	.097	($\pm$0.025)	5.8	7
	14.0	1.09	.037	(5.160)		
	15.0	1.17	.068			
$+NMeEt_2$	6.7	1.43×10^5	5 .155			
	10.3	2.35	.371			
	10.0	2.27	.356	(5.273)		
	10.2	2.31	.364	5.351		
	8.5	1.88	.274	($\pm$0.033)	7.6	8
	12.0	2.76	.441	(5.429)		
	11.2	2.56	.480			
	12.0	2.76	.441			
$+NEt_3$	13.3	6.14×10^5	5 .788			
	13.7	6.32	.801			
	14.1	6.55	.816	(5.765)		
	13.4	6.21	.793	5.785	1.8	7
	12.4	5.70	.756	$\pm$0.008		
	12.4	5.70	.756	(5.805)		
	13.2	6.12	.787			

TABLE III (Contd.)

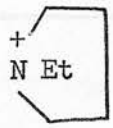
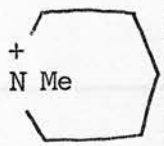
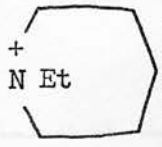
$+NR_3$	Dose-ratio from each preparation	K_B	Log K_B	Mean log K_B ($\pm$S.E.) with 95% Fiducial Limits	Standard error as per cent. of mean	No. of Results
	11.4	12.9×10^4	4 1.111			
	12.0	13.8	1.410			
	14.0	16.8	1.225	(4.989)		
	9.6	10.7	1.029	5.084		
	8.5	9.3	.968	($\pm$0.039)	9	7
	12.5	14.5	1.161	(5.179)		
	8.0	9.0	.954			
	9.2	4.09×10^5	5 .612			
	9.3	4.13	.616			
	7.1	3.05	.484			
	8.4	3.70	.568	(5.535)		
	8.5	3.73	.572	5.568	3	8
	8.3	3.64	.561	($\pm$0.014)		
	8.7	3.84	.584	(5.601)		
	8.0	3.52	.546			
	5.0	2.08×10^5	5 .318			
	8.3	1.82	.260			
	5.7	1.17	.068	(5.116)		
	9.7	1.44	.158	5.194	7.4	7
	10.3	1.56	.193	($\pm$0.032)		
	9.0	1.33	.124	(5.272)		
	11.4	1.74	.240			
	7.9	3.46×10^5	5 .539			
	8.0	3.53	.548			
	8.2	3.59	.555	(5.489)		
	7.0	3.01	.479	5.525	3.5	8
	7.4	3.22	.508	($\pm$0.015)		
	8.9	3.92	.593	(5.560)		
	7.2	3.10	.491			
	7.2	3.07	.487			

TABLE IV

CYCLOHEXYLACETOXYETHYL SERIES

$+NR_3$	Dose-ratio from each preparation	K_B	Log K_B	Mean log K_B ($\pm$S.E.) with 95% F. L.	Standard error as percent of mean	No. of Results
$+NMe_3$	8.8	9.69×10^4	4 .986			
	9.8	10.93	1.039			
	10.0	11.30	1.053			
	8.5	9.30	.969	(4.927)		
	8.1	8.82	.946	4.965		
	6.9	7.38	.868	($\pm$0.017)	3.9	10
	8.3	9.14	.961	(5.003)		
	7.7	8.38	.923			
	7.9	8.66	.938			
	76.6	9.44	.975			
$+NMe_2Et$	22.0	2.63×10^5	5 .420			
	26.3	3.16	.500			
	12.2	2.80	.447			
	12.0	2.76	.441	(5.435)		
	13.7	3.18	.502	5.486		
	13.8	3.26	.513	($\pm$0.022)	5	9
	16.3	3.82	.582	(5.537)		
	15.9	3.73	.572			
	101.0	2.50	.398			
$+NMeEt_2$	13.7	3.18×10^5	5 .502			
	13.7	3.16	.500			
	13.1	3.03	.481	(5.473)		
	16.7	3.91	.592			
	11.8	2.71	.433	5.511	3.7	8
	14.0	3.27	.515	($\pm$0.016)		
	15.1	3.53	.548	(5.549)		
	14.1	3.28	.516			
$+NEt_3$	20.4	4.85×10^5	5 .686			
	17.0	3.99	.601			
	17.1	4.03	.605			
	14.0	3.26	.513	(5.506)		8
	11.3	2.56	.408	5.589	8	
	17.5	4.13	.616	($\pm$0.035)		
	15.6	3.64	.561	(5.672)		
	22.0	5.24	.719			

means of the log affinity lie between 5.0 and 5.6. The standard errors were found to lie between 4-8 per cent. The effects of changes in structure on the affinity of these compounds are very similar to the effects on affinity of those of the cyclohexylpentyl series, even though the esters have lower affinity.

5. The diphenylpentyl series (Table V)

The affinity constants were calculated from experiments in which the dose-ratio was 10-85. The means of the log affinity lie between 6.6 - 7.3 and the standard errors of the estimates for each member of the series lie between 3-9 per cent. The most striking feature of this series is that the affinity rises significantly up to the methyldiethyl compound but with further ethylation, as with triethyl, the affinity falls significantly, even below that of the trimethyl compound. Moreover, contrary to the phenylpentyl series, the affinity goes up with the methylpyrrolidinium compound and with the remaining members of the series it gradually declines till it reaches the lowest value for the series with the ethylpiperidinium compound (Fig. IV, page 48).

6. The diphenylethoxyethyl series (Table VI)

The affinity constants were calculated from the experiments in which the dose-ratio was about 10. The mean values of the log affinity lie between 6.0 and 6.6.

TABLE V DIPHENYLPENTYL SERIES

$+NR_3$	Dose-ratio from each preparation	K_B	Log K_B	Mean log K_B (± S.E.) with 95% Fiducial Limits	Standard error as per cent. of mean	No. of Results
$+NMe_3$	45.5	8.89×10^6	6 .949			
	36.8	9.10	.959			
	48.3	9.85	.993	(6.852)		
	10.0	7.60	.881	6.950		7
	54.5	8.90	.949	(±0.040)	9.2	
	11.9	9.05	.957	(7.048)		
	55.0	9.20	.964			
$+NMe_2Et$	85.7	2.15×10^7	7 .332			
	85.5	2.15	.332	(7.174)		
	65.3	1.64	.215	7.257	6.9	5
	61.0	1.50	.176	(±0.030)		
	17.9	1.69	.228	(7.340)		
$+NMeEt_2$	20.4	9.88×10^6	6 .995			
	58.0	14.40	1.158	(7.019)		
	54.9	13.70	1.133	7.108	7.4	5
	13.6	12.55	1.100	(±0.032)		
	12.2	14.10	1.149	(7.197)		
$+NEt_3$	19.8	4.77×10^6	6 .679			
	20.3	4.91	.691	(6.673)		
	20.0	4.99	.698	6.712	3.2	5
	12.3	5.60	.748	(±0.014)		
	12.0	5.57	.746	(6.751)		

TABLE V (Contd.)

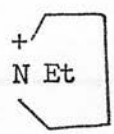
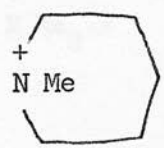
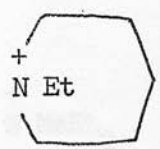
$+NR_3$	Dose-ratio from each preparation	K_B	Log K_B	Mean log K_B ($\pm$S.E.) with 95% Fiducial Limits	Standard error as per cent. of mean	No. of Results
	25.3	7.84×10^6	6 .894	(6.787)		
	31.0	9.50	.978	6.898	8.0	4
	33.4	8.10	.909	($\pm$0.035)		
	20.4	6.46	.810	(7.009)		
	29.0	7.78×10^6	6 .891			
	26.5	6.36	.804			
	17.0	8.10	.909	(6.790)		
	12.6	5.82	.765	6.852	5.3	6
	70.0	6.91	.840	(0.024)		
	17.0	7.97	.902	(6.914)		
	59.7	4.15×10^6	6 .618			
	35.6	5.76	.753	(6.606)		
	27.0	4.37	.641	6.670	5.3	5
	15.0	4.66	.668	($\pm$0.023)		
	15.0	4.66	.668	(6.734)		
	10.9	3.30×10^6	6 .519			
	13.7	4.20	.623			
	10.2	3.07	.487	(6.536)		
	14.9	4.65	.668			
	12.8	3.93	.594	6.595	5.7	8
	13.7	4.24	.627	($\pm$0.025)		
	15.8	4.93	.693	(6.654)		
	11.7	3.55	.550			

TABLE VI

DIPHENYLETHOXYETHYL SERIES

$+NR_3$	Dose-ratio from each preparation	K_B	Log K_B	Mean log K_B ($\pm$S.E.) with 95% Fiducial Limits	Standard error as per cent of mean	No. of Results
$+N\text{-}Me_3$	10.2	2.30×10^6	6 .362			
	10.5	2.38	.377			
	9.9	2.23	.348	(6.368)		
	13.9	3.23	.509			8
	11.5	2.63	.420	6.413 ($\pm$0.019)	4.4	
	12.9	2.97	.473			
	10.8	2.45	.389	(6.458)		
	11.6	2.65	.423			
$+N\,Me_2Et$	11.2	5.10×10^6	6 .708			
	9.4	4.18	.621			
	8.8	3.92	.593	(6.646)		
	12.0	5.49	.740	6.693	4.7	8
	11.0	5.02	.701	($\pm$0.020)		
	12.4	5.76	.760	(6.740)		
	11.5	5.27	.722			
	10.9	4.96	.696			
$+N\,MeEt_2$	9.4	4.21×10^6	6 .624			
	8.1	3.54	.549			
	7.8	3.38	.529	(6.497)		
	7.0	2.98	.474	6.540		
	9.0	3.97	.599	($\pm$0.018)	4	8
	8.3	3.63	.560	(6.583)		
	7.2	3.10	.491			
	7.6	3.28	.516			
$+N\,Et_3$	7.9	2.30×10^6	6 .362			
	6.8	1.95	.290			
	8.7	2.56	.408	(6.315)		
	8.5	2.50	.398	6.374	5.5	7
	10.0	3.02	.480	($\pm$0.024)		
	8.2	2.38	.377	(6.433)		
	7.0	2.01	.303			

TABLE VI (Contd.)

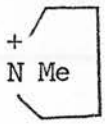
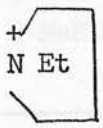
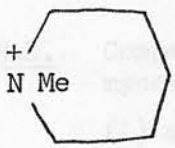
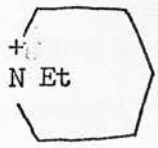
$+NR_3$	Dose-ratio from each preparation	K_B	Log K_B	Mean log K_B (±S.E.) with 95% Fiducial Limits	Standard error as per cent. of mean	No. of Results
	12.8	2.96×10^6	6 .471			
	15.0	3.50	.544			
	17.0	4.00	.602			
	11.0	2.56	.408	(6.437)		
	9.0	2.59	.413	6.508	6.9	8
	10.0	2.94	.468	(±0.030)		
	10.73	3.24	.511	(6.579)		
	14.2	4.40	.644			
	8.0	3.58×10^6	6 .554			
	23.0	5.53	.743			
	8.0	3.58	.554	(6.523)		
	8.2	3.61	.556	6.589		
	8.7	3.84	.584	(±0.028)	6.4	8
	7.2	3.06	.486	(6.655)		
	8.4	3.68	.566			
	10.2	4.62	.665			
	10.7	1.62×10^6	6 .210			
	9.6	1.49	.173			
	9.3	1.38	.140	(6.131)		
	8.2	1.21	.083	6.182	4.8	7
	10.8	1.63	.212	(±0.021)		
	10.5	1.59	.201	(6.233)		
	11.8	1.80	.255			
	6.0	1.30×10^6	6 .114			
	6.2	1.32	.121			
	10.0	1.50	.176	(6.130)		
	9.8	1.46	.164	6.151	2	8
	9.2	1.36	.134	(±0.009)		
	10.3	1.54	.188	(6.172)		
	9.7	1.46	.164			
	9.5	1.41	.149			

TABLE VIA

DI PHENYLETHOXYETHYL SERIES (SCOTT'S RESULTS)

$+NR_3$	Dose-ratio from each preparation	K_B	Log K_B	Mean log K_B (±S.E.) with 95% Fiducial Limits	Standard error as per cent. of mean	No. of Results
$+NMe_3$		2.61×10^6	6 .417			
		2.73	.436	(6.412)		
		2.64	.423	6.425	0.9	4
		2.65	.423	(±0.004)		
				(6.438)		
$+NMe_2Et$		4.56×10^6	6 .659	(6.604)		
		4.32	.636	6.699		
		5.78	.762	(±0.030)	6.9	4
		5.47	.738	(6.794)		
$+NMeEt_2$		4.71×10^6	6 .673	(6.621)		
		5.03	.702	6.669		
		4.25	.628	(±0.015)	3.5	4
		4.69	.671	(6.717)		
$+NEt_3$		3.15×10^6	6 .498			
		3.15	.498	(6.490)		
		3.15	.498	6.497	0.6	5
		3.19	.504	(±0.002)		
		3.07	.487	(6.504		

N.B. Comparison between the mean log affinity constant obtained by Scott and myself shows that -

(1) mean values for $R'^{+}NMe_3$ and $R'^{+}NMe_2Et$ are not significantly different at a Probability level of 0.05.

(2) Scott obtained the mean values of $R'^{+}NMeEt_2$ and $R'^{+}NEt_3$ which are significantly higher than those obtained by me, at a Probability level of 0.001.

The standard errors were only within 2-7 per cent. The effects of changes in structure on the affinity of these compounds are very similar to the effects of changes of those of the diphenylpentyl series, even though the ethers have a 'lower' affinity.

The first four members of this series were also tested, in a slightly different method by Scott (1962), and the dose-ratios were calculated graphically. The values for the affinity constants were shown in Table VIA for comparison.

The results obtained by me for trimethyl and ethyldimethyl ammonium compounds do not significantly differ from those obtained by Scott at a probability level of 0.05. However, the results obtained by me for the methyldiethyl and triethyl ammonium compounds are significantly lower than those obtained by him, even at a probability level of 0.001.

7. <u>The phenylcyclohexyl acetoxyethyl series</u> (Table VII)

The affinity constants were calculated from experiments in which the dose-ratios lie between 10-40. The affinity of these compounds have the highest values amongst the acetylcholine analogues tested. The mean values of the log affinity lie between 8.6 and 9.0. The standard errors of estimates for each member of the series lie within 2-9 per cent. The most important feature of this series is that with the introduction

TABLE VII

PHENYLCYCLOHEXYLACETOXYETHYL SERIES

$+NR_3$	Dose-ratio from each preparation	K_B	Log K_B	Mean log K_B ($\pm$S.E.) with 95% Fiducial Limits	Standard error as per cent. of mean	No. of Results
$+NMe_3$	120.8	3.02×10^8	8 .480			
	12.0	2.69	.430			
	12.0	2.81	.449			
	15.0	3.53	.548	(8.445)		
	15.0	3.57	.553	8.504	5.5	7
	13.0	2.94	.468	($\pm$0.024)		
	16.0	3.98	.600	(8.563)		
$+NMe_2Et$	38.2	9.30×10^8	8 .969			
	36.1	8.78	.944			
	38.3	9.33	.970			
	39.6	9.89	.995	(8.867)		
	34.7	8.43	.926	8.890	2.3	9
	26.5	6.37	.804	($\pm$0.010)		
	23.7	5.68	.754	(8.913)		
	33.7	8.18	.913			
	24.3	5.82	.765			
$+NMeEt_2$	18.5	4.37×10^8	8 .641			
	31.2	7.55	.878			
	21.4	5.09	.707	(8.676)		
	24.7	5.93	.773	8.771	9.0	7
	29.7	7.18	.856	($\pm$0.039)		
	31.6	7.64	.883	(8.866)		
	19.0	4.54	.657			
$+NEt_3$	15.3	3.58×10^8	8 .554			
	16.7	3.92	.593			
	15.4	3.59	.555	(8.521)	4.4	8
	19.4	4.60	.663	8.566		
	14.5	3.38	.529	($\pm$0.019)		
	15.0	3.48	.542	(8.611)		
	17.2	4.05	.608			
	13.2	3.04	.483			

TABLE VII (Contd.)

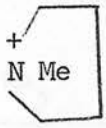
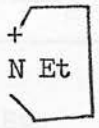
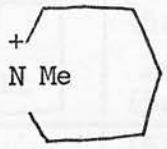
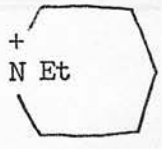
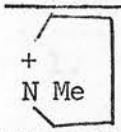
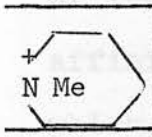
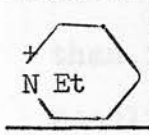
+ NR_3	Dose-ratio from each preparation	K_B	Log K_B	Mean log K_B (±S.E.) with 95% Fiducial Limits	Standard error as per cent. of mean	No. of Results
	12.0	2.70×10^8	8 .431			
	17.4	4.09	.612			
	16.0	3.74	.573	(8.501)		
	20.7	4.92	.692	8.574	6.9	7
	15.0	3.54	.549	(±0.030)		
	16.6	3.90	.591	(8.647)		
	15.7	3.69	.567			
	17.0	4.02×10^8	8 .604			
	19.0	4.47	.650	(8.599)		
	20.0	4.78	.679	8.650	4.6	6
	19.2	4.56	.659	(±0.020)		
	16.5	3.89	.590	(8.701)		
	22.5	5.37	.730			
	9.7	2.18×10^8	8 .339			
	20.7	2.46	.391	(8.246)		
	18.2	2.15	.332	8.310	5.8	6
	14.3	1.66	.220	(±0.025)		
	15.5	1.81	.258	(8.374)		
	17.9	2.11	.324			
	36.7	1.18×10^8	8 .072			
	8.6	1.08	.033			
	8.9	1.12	.049	(8.052)		
	8.9	1.13	.053			
	11.6	1.51	.179	8.111	5.8	8
	13.0	1.73	.238	(±0.025)		
	10.7	1.39	.143	(8.170)		
	293.0	1.33	.124			

TABLE VIIA

Summary of results for acetylcholine analogues.

Mean log values for affinity with 95% Fiducial limits in parentheses.

1	2	3	4	5	6	7	8
$+NR_3$ R	Ph-Pentyl	Cyclo hexyl-pentyl	Ph acetoxy-ethyl	Cyclo hexyl acetoxy-ethyl	Ph_2Pentyl	Ph_2ethoxy-ethyl	Ph cyclohexyl acetoxy ethyl
$+N\,Me_3$	(5.141) 5.179 (5.217)	(5.339) 5.411 (5.483)	(4.504) 4.533 (4.562)	(4.927) 4.965 (5.003)	(6.852) 6.950 (7.048)	(6.368) 6.413 (6.458)	(8.445) 8.504 (8.563)
$+N\,Me_2Et$	(5.379) 5.446 (5.513)	(5.761) 5.825 (5.889)	(5.038) 5.099 (5.160)	(5.435) 5.486 (5.537)	(7.174) 7.257 (7.340)	(6.646) 6.693 (6.740)	(8.867) 8.890 (8.913)
$+N\,MeEt_2$	(5.683) 5.714 (5.745)	(5.789) 5.853 (5.917)	(5.273) 5.351 (5.429)	(5.473) 5.511 5.549	(7.019) 7.108 (7.197)	(6.497) 6.540 (6.583)	(8.677) 8.771 (8.866)
$+N\,Et_3$	(5.875) 5.894 (5.913)	(5.835) 5.922 (6.009)	(5.765) 5.785 (5.805)	(5.506) 5.589 (5.672)	(6.673) 6.712 (6.751)	(6.315) 6.374 (6.433)	(8.521) 8.566 (8.611)
	(5.588) 5.632 (5.676)	(5.584) 5.702 (5.820)	(4.989) 5.084 (5.179)		(6.787) 6.898 (7.009)	(6.437) 6.508 (6.579)	(8.501) 8.574 (8.647)
	(5.553) 5.631 (5.709)	(5.768) 5.814 (5.860)	(5.535) 5.568 (5.601)		(6.790) 6.852 (6.914)	(6.523) 6.589 (6.655)	(8.599) 8.650 (8.701)
	(5.643) 5.684 (5.725)	(5.855) 5.919 (5.983)	(5.116) 5.194 (5.272)		(6.606) 6.670 (6.734)	(6.131) 6.182 (6.233)	(8.246) 8.310 (8.374)
	(5.412) 5.456 5.500	(5.917) 6.025 (6.133)	(5.489) 5.525 (5.560)		(6.536) 6.595 (6.654)	(6.130) 6.151 (6.172)	(8.052) 8.111 (8.170)

of both the benzene and cyclohexane rings together in the acetyl moiety the affinity has increased by about 10,000 fold when compared with the affinity of the compounds containing a single benzene or cyclohexane ring. Although the affinity for the series is higher than the diphenylpentyl and diphenylethoxyethyl series, the effect of changes in structure on affinity are similar to the effect on affinity of those of the diphenylpentyl and diphenylethoxyethyl series.

The affinity constants for the atropine analogues

The affinity constants of the three series of tropine derivatives, for the guinea-pig ileum, are shown in Tables VIII - XIII and summarised in Table XIV.

1. The benziloyltropine alkyl iodides (Tables VIII and IX)

The affinity of the members of this series are the highest of all the compound tested. The mean values of log affinity constants lie between 8.0 and 10.5. The affinity constants were calculated from experiments in which, usually, the dose-ratios were comparatively higher than in the other series. This did not affect the result, as can be seen from the graph shown in Fig. II (page 26). The standard errors were within 4-8 per cent.

The results show that the values for the ethylated homologues were significantly lower than the methylated

TABLE VIII

BENZILOYL-TROPINE ALKYLIODIDES

$^{+}_{NR}$	Dose-ratio from each Preparation	K_B	Log K_B	Mean log K_B ($\pm$S.E.) with 95% Fiducial Limits	Standard error as per cent: of mean	No. of Results
	5130.0	4.15×10^{10}	10 .618			
	4325.0	3.49	.543			
Me	369.0	2.97	.473			
	1053.0	3.13	.495			
	1175.0	3.49	.543	(10.363)		
	233.0	1.97	.295	10.443	8.3	11
	350.0	2.08	.318	($\pm$0.036)		
	1125.0	3.34	.524	(10.523)		
	1025.0	3.05	.484			
	668.0	1.98	.297			
	3195.0	1.90	.279			
	38.0	1.64×10^{9}	9 .215			
	26.5	1.09	.037			
	29.6	1.22	.086			
Et	33.0	1.38	.140	(9.061)		
	29.0	1.22	.086	9.100	3.9	9
	30.0	1.24	.093	($\pm$0.017)		
	26.5	1.09	.037	(9.139)		
	30.4	1.26	.100			
	-	1.28	.107			

TABLE IX

BENZILOYL PSEUDO TROPINE ALKYLIODIDES

$\overset{+}{N}R$	Dose-Ratio from each Preparation	K_B	Log K_B	Mean log K_B ($\pm$S.E.) with 95% Fiducial limits	Standard error as per cent. of mean	No. of Results
Me	816.0	7.02×10^9	9 .846			
	465.0	5.49	.740			
	468.0	5.50	.740	(9.765)		
	-	6.64	.822	9.819 ($\pm$0.022)	5.0	7
	629.0	7.40	.869			
	-	7.88	.896	(9.873)		
	336.0	6.59	.819			
Et	19.0	17.10×10^7	7 1.233			
	10.4	9.17	.962			
	12.3	9.94	.997	(7.993)		
	14.0	11.66	1.067	8.088 ($\pm$0.039)	9	7
	19.8	16.15	1.206			
	18.4	12.36	1.093	(8.183)		
	17.2	11.50	1.061			

ones, in both the tropine and pseudotropine derivatives at a probability level of 0.001. The affinity of the pseudotropine derivativeswas significantly lower than those of the tropine derivatives at a probability level of 0.001.

2. The diphenyl acetyl tropine alkyl iodides (TablesX&XI)

Experiments from which the affinity constants were calculated for the methylated homologues had a higher dose-ratio than for the ethylated ones, in both tropine and pseudotropine derivatives. The mean values of log affinity constants lie between 6.9 and 8.7. The standard errors fall between 4-12 per cent. The effect of changes in structure on affinity are similar to those in the benziloyl tropine series.

3. Tropine alkyl iodides (Tables XII and XIII)

Experiments from which the affinities were calculated had a very low dose-ratio. These compounds have the lowest affinity amongst the compounds tested. The mean values of log affinity constants lie at about 3.0. The results show no significant difference amongst the members of the series at a probability level of 0.05.

The test for competitive antagonism (Table XV)

A few of the compounds of the acetylcholine analogues were selected at random to see if they were

TABLE X DIPHENYL ACETYL TROPINE ALKYLIODIDES

±NR	Dose-ratio from each Preparation	K_B	Log K_B	Mean log K_B (±S.E.) with 95% Fiducial limits	Standard error as per cent. of mean	No. of Results
Me	466.0	4.67×10^8	8 .669	(8.618) 8.669 (±0.02) (8.720)	4.6	6
	147.0	4.41	.644			
	-	5.29	.723			
	129.0	3.85	.586			
	159.0	4.75	.677			
	173.0	5.18	.714			
Et	19.7	8.50×10^7	7 .931	(7.789) 7.864 (±0.029) (7.939)	6.7	6
	20.7	9.00	.954			
	18.0	7.76	.890			
	16.0	6.83	.834			
	14.0	6.03	.780			
	14.7	6.24	.795			

TABLE XI DIPHENYLACETYL PSEUDO TROPINE ALKYLIODIDES

+ NR	Dose-ratio from each Preparation	K_B	Log K_B	Mean log K_B (±S.E.) with 95% Fiducial limits	Standard error as per cent. of mean	No. of Results
Me	129.0	1.34×10^8	8 .127	(8.007)	12	3
	185.0	1.93	.286	8.231 (±0.052)		
	183.0	1.91	.281	(8.455)		
Et	18.2	9.00×10^6	6 .954	(6.775)	9	6
	16.0	7.73	.888			
	10.4	4.93	.693	6.875 (±0.039)		
	63.0	6.94	.841			
	16.7	8.30	.919	(6.975)		
	18.0	8.89	.949			

TABLE XII

TROPINE ALKYLIODIDES

$^{+}_{NR}$	Dose-ratio from each Preparation	K_B	Log K_B	Mean log K_B ($\pm$S.E.) with 95% Fiducial limits	Standard error as per cent. of mean	No. of Results
Me	3.75	1.37×10^{3}	3 .137			
	2.30	1.65	.218	(3.085)		
	2.50	1.81	.258	$\underline{3.166}$ ($\pm$0.039)		
	2.23	1.53	.185		9.0	5
	3.15	1.08	.033	(3.274)		
Et	4.4	1.69×10^{3}	3 .228			
	4.2	1.62	.210	(3.062)		
	4.1	1.52	.182	$\underline{3.162}$ ($\pm$0.036)	8.3	5
	4.0	1.47	.167			
	3.1	1.06	.025	(3.262)		

TABLE XIII

PSEUDO TROPINE ALKYLIODIDES

$^{+}_{NR}$	Dose-ratio from each Preparation	K_B	Log K_B	Mean log K_B ($\pm$S.E.) with 95% Fiducial limits	Standard error as per cent of mean	No. of Results
Me	2.8	1.11×10^{3}	3 .045			
	3.3	1.41	.149	(3.065)		
	3.5	1.56	.193	$\frac{3.142}{(\pm 0.030)}$	6.9	6
	3.7	1.70	.230			
	2.9	1.16	.065	(3.219)		
	3.4	1.48	.170			
Et	3.4	1.20×10^{3}	3 .079			
	3.8	1.38	.140	(3.086)		
	3.8	1.37	.137	$\frac{3.142}{(\pm 0.020)}$	4.6	6
	3.9	1.43	.155			
	4.2	1.59	.201	(3.198)		

TABLE XIV

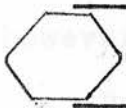
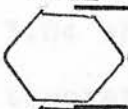
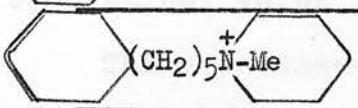
SUMMARY OF RESULTS FOR THE ATROPINE ANALOGUES

MEAN LOG AFFINITY CONSTANTS WITH 95% CONFIDENCE LIMITS

"Body"	TROPINE		PSEUDO TROPINE	
	Meth	Eth	Meth	Eth
Benziloyl	(10.363) 10.443 (10.523)	(9.061) 9.100 (9.139)	(9.765) 9.819 (9.873)	(7.993) 8.088 (8.183)
Diphenyl acetyl	(8.618) 8.669 (8.720)	(7.789) 7.864 (7.939)	(8.007) 8.231 (8.455)	(6.775) 6.875 (6.975)
Hydroxyl	(3.058) 3.166 (3.274)	3.062 3.162 (3.262)	(3.065) 3.142 (3.219)	(3.086) 3.142 (3.198)

TABLEXV

Summary of results; for test for antagonism.

1	2	3	4	5
Compounds	Dose-ratio for atropine alone	Dose-ratio for Comp. alone	Observed D-R for 2 + 3 together	Calculated Dose-ratio
	107.0	10.41	1.44	1.09
	87.0	16.43	1.37	1.15
	132.0	13.14	1.34	1.17
	112.0	10.74	1.50	1.09
$Ph(CH_2)_5\overset{+}{N}$-Et	103.0	18.4	1.12	1.17
$Ph_2CH(CH_2)_4\overset{+}{N}$-$Me_3$	102.0	10.67	1.18	1.09
$Ph_2CH(CH_2)_4\overset{+}{N}$-$MeEt_2$	116.0	12.55	1.26	1.11
$Ph_2CH(CH_2)_4\overset{+}{N}$-$Et_3$	91.0	12.22	1.29	1.11
$Ph_2CH(CH_2)_4\overset{+}{N}$-Me	113.0	12.50	1.08	1.11
"	99.0	10.0	1.15	1.09
$Ph_2CH(CH_2)_4\overset{+}{N}$-Me	82.0	15.0	1.16	1.13
Papaverine	96.4	4.06	3.84	1.03

NB. The mean dose-ratio for atropine of 104.0 was used to calculate the value for column 5.

competitive antagonists and were tested by the atropine method. The results are summarised in Table XV and show a fair agreement between the observed and theoretical values for the compounds.

The observed mean values for 11 compounds was 1.26 and the theoretical mean value was 1.11. These means, however, differ from each other at probability of 0.005.

Papaverine (4.0×10^{-5} M) produced a dose-ratio of 4.06 alone on guinea-pig ileum and it produced a dose-ratio of 3.84 when combined with atropine (10^{-7} M), whereas, the theoretical value for competition is only 1.03.

The mean dose-ratio for atropine (10^{-7} M) was 104.0 and this value was used to calculate the theoretical values. The mean value of affinity of atropine for the guinea-pig ileum was $10^{9.009}$ (1.023×10^{9}) litre/mole.

The effect of temperature on affinity

Table XVI summarises the effects of temperature on the affinity constants. The mean value of log affinity constant at 27°C and 37°C were found to be 5.396 and 5.213 respectively. The results indicate that with the rise of temperature the affinity decreases significantly. The mean values are significantly different at a probability level of 0.001.

From these two values of affinity the difference in enthalpy, ΔH, and the entropy, ΔS, can be calculated after Dixon and Webb (1964).

TABLE XVI

Effect of temperature on the affinity constant of Phenylacetoxyethylmethyl piperidinium, on the guinea-pig ileum.

Serial No.	27°C		37°C	
	individual estimates of K_b	log K_b	individual estimates of K_b	log K_b
1	2.96×10^5	5.471	1.93×10^5	5.286
2	3.65	.502	1.54	.188
3	2.79	.446	1.26	.100
4	2.86	.456	1.50	.176
5	2.30	.362	1.71	.233
6	2.91	.262	1.60	.243
7	2.26	.354	1.75	.243
8	2.53	.403	1.79	.253
9	2.39	.378	1.91	.281
10	1.72	.236	1.39	.248
11	2.42	.388	1.77	.248
12	1.94	.288	1.43	.155
13	2.22	.346	1.80	.255
Mean ± s.e.	2.49×10^5 -	5.396 ± 0.023	1.63×10^5 -	5.213 ± 0.016
95% Confidence limits		(5.346) (5.446)		(5.178) (5.248)

The means are significantly different at a probability level of 0.05 ($P < 0.001$).

Temperature Co-efficient (Q_{10}) = 5.396 - 5.213 = 0.183 and taking antilog, it becomes 1.524.

(1) $\Delta F_T = -2.303\ R\ T \log \frac{K_B}{1}$ cal/mole.

$\Delta F_{T300} = -7404.00$ cal/mole.

$\Delta F_{T310} = -7391.33$ cal/mole.

(2) $\Delta H = 2.303\ R\ T^2 \frac{d \log K_B}{d\ T}$ cal/mole.

$\Delta H = -8884.28$ cal/mole.

(3) $\Delta S = \frac{\Delta H - \Delta F}{T}$ cal/deg.

$\Delta S = -4.816$ cal/deg.

Paton and Rang (1966) reported similar results.

The estimation of affinity constants, using different agonists

The affinity constants of some of the antagonists were measured using different agonists. The results are shown in Tables XVII-XIX. The affinity constants of some of the members of the phenylpentyl series were determined using acetylcholine and carbachol as agonists. At a probability level of 0.05 there was no significant difference between the mean values obtained with different agonists. The affinity of phenylpentylethyl-pyrrolidinium was determined using carbachol and pentyl-trimethyl ammonium on the same piece of guinea-pig ileum, in one group of experiments, and carbachol and ethoxy-ethyltrimethyl ammonium, in the same piece of ileum, in another group of experiments. The results are shown in

TABLE XVIIA

Log affinity constants of Phenylpentyl trimethyl ammonium on guinea pig ileum with acetylcholine and carbachol as agonists. The values are from single piece of tissue.

	Acetylcholine	Carbachol	Remarks
Individual Values	5.196	5.170	
	.107	.179	
	.161	.100	
	.228	.146	
	.253	.253	
Mean ± s.e.	5.189	5.170	Means are not significantly different at a probability level of 0.05.
	±0.024	±0.024	
95% Confidence Limits	(5.122)	(5.103)	
	(5.256)	(5.237)	

TABLE XVIIB

Log affinity constant of Phenylpentyl diethyl methyl ammonium on guinea pig ileum with acetylcholine and carbachol as agonists. The values obtained from different pieces of tissues.

	Acetycholine	Carbachol	Remarks
Individual Values	5.672 .740 .762 .693 .663 -	5.762 .699 .729 .782 .627 .725	
Mean ± s.e.	5.710 ±0.061	5.720 ±0.024	Means are not significantly different at a probability level of 0.05.
95% Confidence Limits	(5.540) (5.880)	(5.658) (5.782)	

TABLE XVIIC

Log affinity constant of Phenylpentyl trielhyl ammonium on guinea pig ileum, with acetylcholine and carbachol as agonists. The values obtained from different pieces of tissues.

	Acetylcholine	Carbachol	Remarks
Individual Values	5.889 .914 .853 .929 .925 .903 .914	5.900 .841 .890 .863 .945 .856 -	
Mean ± s.e.	5.900 ±0.010	5.880 ±0.014	Means are not significantly different at a probability level of 0.05.
95% Confidence Limits	(5.876) (5.925)	(5.844) (5.916)	

TABLE XVIII

Log affinity constants of Phenyl-pentylethyl pyrrolidinium, on guinea pig ileum with carbachol and pentyltrimethyl ammonium as agonists. The values obtained from the same piece of tissue.

	Carbachol	Pentyl TMA	Remarks
Individual Values	5.710 .716 .608 .560 .549 .760	5.764 .733 .788 .691 .674 .667	
Mean ± s.e.	5.650 ±0.036	5.720 ±0.020	Means are not significantly different at a probability level of 0.05.
95% Confidence Limits	(5.669 (5.771)	(5.557) (5.743)	

TABLE XIX

Log affinity constant of phenyl pentylethyl pyrrolidinium on guniea pig ileum with carbachol and ethoxyethyl-terimethyl ammonium as agonists. The value obtained from the same piece of tissue.

	Ethoxyethyl TMA	Carbachol	Remarks
Individual Values	5.636 .690 .606 .528 .640	5. - .491 .722 .581	
Mean ± s.e.	5.620 ±0.026	5.598 ±0.067	Means are not significantly different at a probability level of 0.05.
95% Confidence Limits	(5.548) (5.693)	(5.310) (5.886)	

Tables XVIII-XIX. There was, again, no significant difference between the means at a probability level of 0.05.

The estimation of affinity constants for various tissues

To see if there is any variability in affinity constants for different tissues, some of the compounds were tested, on the various tissue-preparations. The values are shown in Tables XX-XXIII.

Table XX shows the results obtained for diphenylacetyltropine ethiodide on guinea-pig ileum, longitudinal strip muscle and taenia coli muscle. There was no significant difference between the three means, at a probability level of 0.05.

However, the means obtained for the phenylcyclohexylacetoxyethyl-trimethylammonium compound on the guinea-pig ileum and taenia coli muscle were found to be significantly different at a probability level of 0.001 (Table XXI).

All the eight members of the phenylacetoxyethyl series were tested on rat colon preparations and on guinea-pig ileum. The mean values are shown in Table XXII. With the exception of the three members, the results show no significant difference at a probability level of 0.05. The affinity constant of the trimethyl compound, on the rat colon, was significantly higher than that on the other tissue at a probability level of 0.005. The

TABLE XX.

Log affinity constants of the compounds on the various tissues.

	1	2	3
	Guinea-pig ileum	Guinea-pig Logitudinal strip muscle	Guinea-pig Tania Coli muscle
Diphenyl Acetyl tropine ethiodide	7.829 .751 .740 .786	7.683 .715 .708 .779	7.772 .734 .636 .648
Mean ± s.e.	7.776 ±0.020	7.721 ±0.020	7.698 ±0.033
95% Confidence Limit	(7.712) (7.840)	(7.657) (7.785)	(7.593) (7.802)
Remarks	-	This mean is not sig-:nificantly different from that of ileum ($0.2 > P > .1$)	This mean is not sig-:nificantly different from that of ileum ($0.6 > P > 0.5$)

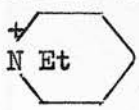
TABLE XXI

Log affinity constants of the compound on the different tissue preparations.

	Guinea-pig ileum	Guinea-pig taenia coli
Phenyl, cylohexyl acetoxyethyl tri methyl ammonium	8.554	8.436
	.593	.474
	.555	.423
	.663	.352
	.529	-
	.542	-
	.608	-
	.483	-
Mean ± S.e	8.566	8.421
	±0.019	±0.025
95% Confidence Limit	(8.521)	(8.341)
	(8.611)	(8.500)
Remark	The means are different at probability of 0.05. (P<0.001)	

TABLE XXVII

Log affinity constants of Phenylacetoxethyl series on the guinea-pig ileum and Rat colon.

$+NR_3$	Guinea-pig ileum. mean log K_b $\pm$ s.e.	Rat colon. mean log K_b $\pm$ s.e.	Value of t	Level of p	Degrees of Freedom	Remarks, significant when $P<0.05$
$+NMe_3$	4.514 $\pm$ 0.024 (7)	4.675 $\pm$ 0.030 (6)	4.35	$0.005>P>.001$	11	significant ($P<0.05$)
$+NMe_2Et$	5.093 $\pm$ 0.024 (7)	5.111 $\pm$ 0.017	0.60	$0.6>P>0.5$	11	
$+NMeEt_2$	5.350 $\pm$ 0.030 (8)	5.320 $\pm$ 0.033 (6)	0.625	$0.6>P>0.5$	12	
$+NEt_3$	5.785 $\pm$0.008 (7)	5.712 $\pm$ 0.036 (5)	1.97	$0.2>P>0.1$	10	
	5.084 $\pm$ 0.039 (6)	5.154 $\pm$ 0.033 (6)	1.4	$0.2>P>0.1$	10	
	5.568 $\pm$ 0.014	5.440 $\pm$ 0.029 (10)	3.88	$0.005>P>.001$	16	significant ($P<0.05$)
	5.194 $\pm$ 0.033	5.282 $\pm$ 0.031 (6)	1.98	$0.1>P>0.05$	10	
	5.528 $\pm$ 0.015 (8)	5.428 $\pm$ 0.030 (6)	3.03	$0.02>P>.01$	12	significant ($P<0.05$)

Figures in the parentheses indicate the number of individual estimations on which the mean is based.

affinity constants of the ethylpyrrolidinium and ethylpiperidinium compounds on the rat colon were significantly lower, at a probability level of 0.005 and 0.02 respectively, than those on the guinea-pig ileum.

Phenylacetoxyethyl-methylpiperidinium and diphenylethoxyethyltrimethyl ammonium compounds were tested on rabbit auricle preparations and guinea-pig ileum. The results are shown in Table XXIII. These mean values show no significant difference at a probability level of 0.05.

TABLE XXIII

Mean log affinity constants of the compounds on the guinea-pig ileum and rabbit auricle.

Compound	Guinea-pig ileum mean log K_b ± s.e.	Rabbit auricle mean log K_b ± s.e.	Value of t	Level of p	Degrees of Freedom	Remarks significance when $P < 0$
$PhcH_2COO(cH_2)_2$	5.194 ± 0.032 (7)	5.222 ± 0.086 (3)	0.304	$0.8 > P > 0.7$	8	not significant
$Ph_2cHcH_2OcH_2$ cH_2 $\overset{+}{N}Et_3$	6.374 ± 0.024 (7)	6.459 ± 0.052 (3)	1.49	$0.2 > P > 0.1$	8	not significant

Figures in the parentheses indicate the number of individual estimates on which the mean is based.

D I S C U S S I O N

Variance of the results

When assessing the scatter of the estimates of affinity constant (K_B), Barlow, Scott and Stephenson (1963) and Abramson (1964) have assumed that there is a normal distribution of estimates of log K_B. This follows from the suggestion by Gaddum (1945,1953) that it is log dose rather than dose which is distributed normally. Barlow, Scott and Stephenson (1963) assumed that their values of the standard error of log K_B were estimates of the variance of log K_B in general about a true value, and therefore used a pooled estimate of the variance of their results with all the compounds, when calculating the fiducial limits of log K_B or of ratios of log K_B.

During the testing of the compounds described above, however, it seemed possible that the variance with some types of compounds might be greater than with others. The fiducial limits of log K_B for the individual compounds (shown in Tables I-XIV) have, therefore, been calculated using the observed variance with the particular compound alone. If there really is a difference in the variance of different types of compounds, it would seem likely that this is related to the chemical nature of the compound and accordingly the variance amongst the series has been calculated and shown in Table XXIV. There are differences amongst the series and there are also differences within a series but it is very difficult

TABLE XXIV

The calculated variances of log. affinity constants for the various series.

Series.	Variance.
Ph-pentyl-	0.00370
Cyclo-Hex-pentyl	0.00840
Ph-acetoxyethyl	0.00442
Cyclo-Hex-acetoxy-ethyl	0.00462
Ph_2-Pentyl	0.00514
Ph_2-ethoxyethyl	0.00378
CycloHex(Ph)-acetoxyethyl	0.00421
	0.00489 (Pooled variance)

to come to any conclusion as to which groups are associated with high variance. The biggest values of the standard error are 11-12 per cent. and most values are less than 10 per cent. and this is very similar to the values obtained by Schild (1947), Barlow, Scott and Stephenson (1963) and by Arunlakshana and Schild (1959). In working out the fiducial limits of the log of ratios of the affinity constants (log K_a/K) a pooled estimate of the variance has been taken, based on all the estimates of log K_B, in the same way as was done by Barlow, Scott and Stephenson (1963). From what has been found in Table XXIV this may lead to an over optimistic value of the fiducial limits in some instances but the limits should not be grossly distorted; for example, the fiducial limits (P = 0.05) of the log $\frac{K_a}{K}$ for cyclohexylpentylethyldimethyl ammonium and trimethyl ammonium are

$$5.825 - 5.411 = 0.414 \pm 0.102$$

with the variance for the series (which is the biggest of all the variances) and

$$0.414 \pm 0.078$$

with the pooled variance.

Different agonists:

The experiments with different agonists (page 41) confirm that the antagonists are blocking the same receptors, and justifies the use of carbachol rather than acetylcholine in the majority of the experiments. The use of carbachol prevents any complications which might arise because the compounds blocked cholinesterases;

there is a real possibility that this may happen, because compounds which resemble acetylcholine enough to block acetylcholine receptors in the ileum may block the destruction of acetylcholine by cholinesterases.

Different tissues:

It is also striking that in general the affinities for the receptors in the guinea-pig ileum, longitudinal strip muscle, taenia coli, rat colon and rabbit auricle are very similar. There are some differences which are significant but none of these is big. The results are similar to those obtained by Arunlakshana and Schild (1959) and Hawkins and Schild (1951) with antihistamine drugs, who found that the affinity of these was the same for histamine receptors in a variety of different tissues.

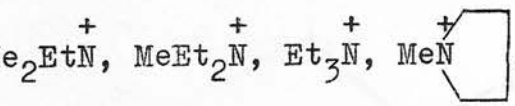
Different temperatures:

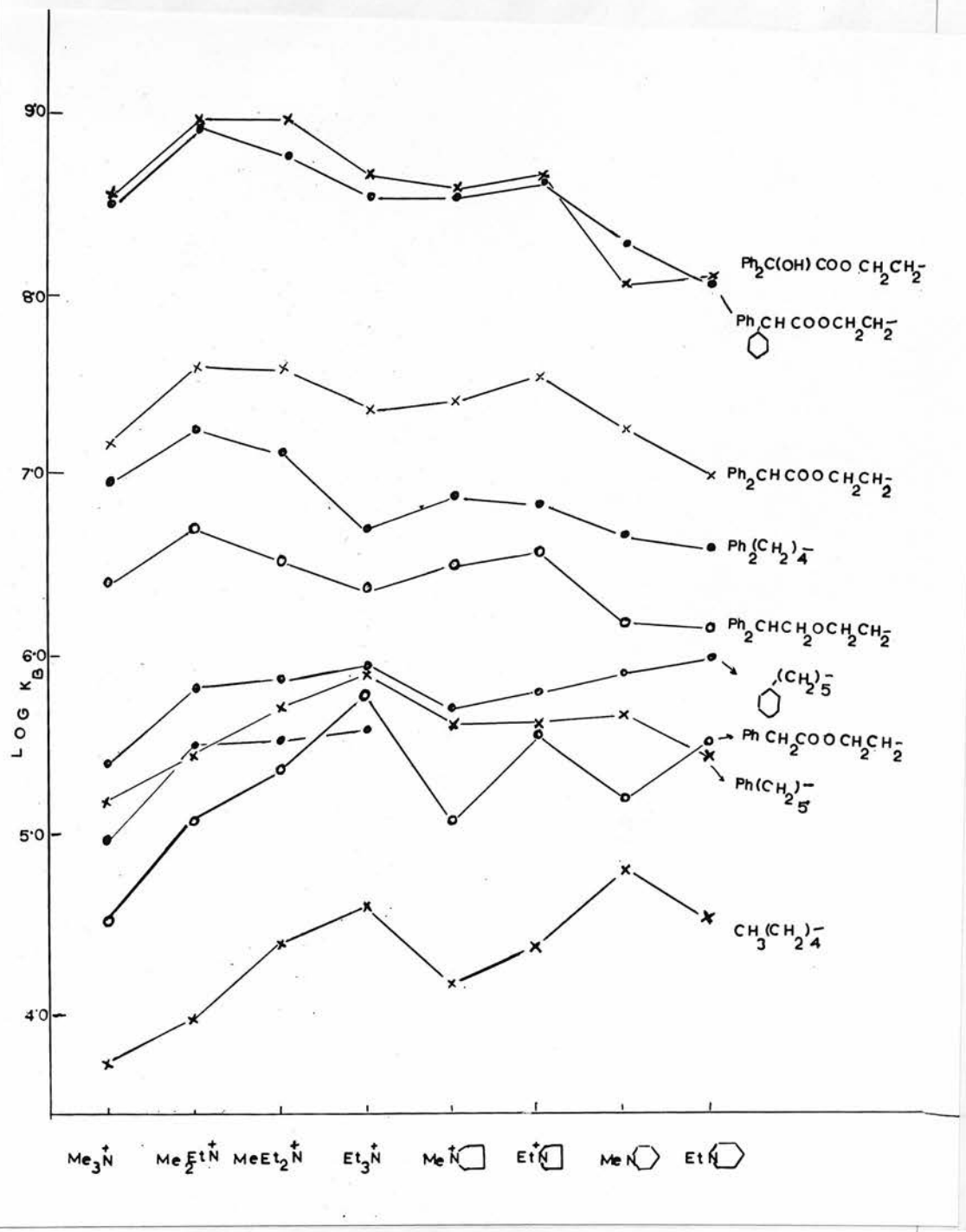
The experiments on affinity at different temperatures indicate a temperature coefficient of 1.5 for the affinity constant of phenylacetoxyethyl-N-methylpiperidinium. This is similar to the coefficient for the binding of many substrates and enzymes (Dixon and Webb, 1964). It indicates that the process of association is exothermic and the process of dissociation is endothermic. It would be desirable to have information about more compounds but unfortunately the measurement of the temperature coefficient for single compound is very laborious and it has not been possible to study them systematically.

Test for competition

The results of the tests for competition justify the

assumption that the compounds are acting competitively in the concentrations at which K_B is estimated. When there was any doubt about the nature of the antagonism, because the graph of (dose-ratio - 1) against antagonist concentration was not linear, the compound was subjected to the atropine test (page 26). All the compounds so tested (Table XV) appeared to be competitive antagonists in the lower concentrations and the estimates of K_B were therefore made in this range. The reliability of the atropine test is shown by the results with papaverine.

The effect of changes in the composition in the onium group is illustrated in Figures IV and V (Tables XXV-XXVII). In the first of these log K is plotted against the composition of the onium group arranged arbitrarily in the order $Me_3\overset{+}{N}$, $Me_2Et\overset{+}{N}$, $MeEt_2\overset{+}{N}$, $Et_3\overset{+}{N}$,  , , and . In the second, an attempt has been made to arrange these groups in order of size, by adding together the atomic weights of the atoms forming the onium groups (omitting the "body" of the molecule). In addition to the compounds tested in this work, results are also included for the benzilic and diphenylacetyl esters studied by Abramson and by Scott and also for n-pentyl compounds studied by Stephenson (unpublished). The first four compounds of the diphenylethoxyethyl series



FIGUREIV. Graph of log affinity (ordinate) and composition of the onium group (abscissa).

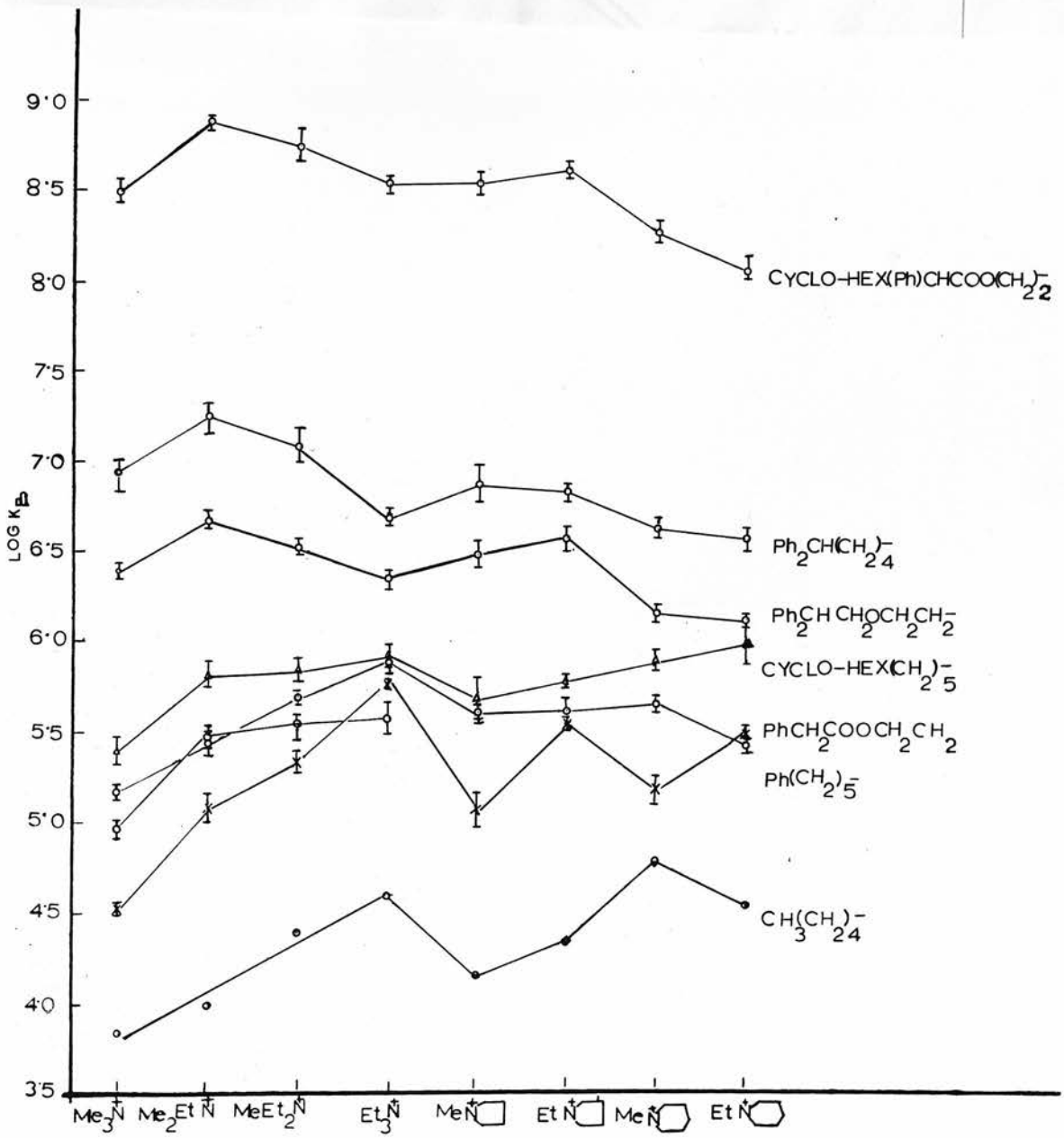


FIGURE 1VA. Graph of log affinity and composition of the onium group. Vertical lines indicate 95 per cent. confidence limits calculated for each compound.

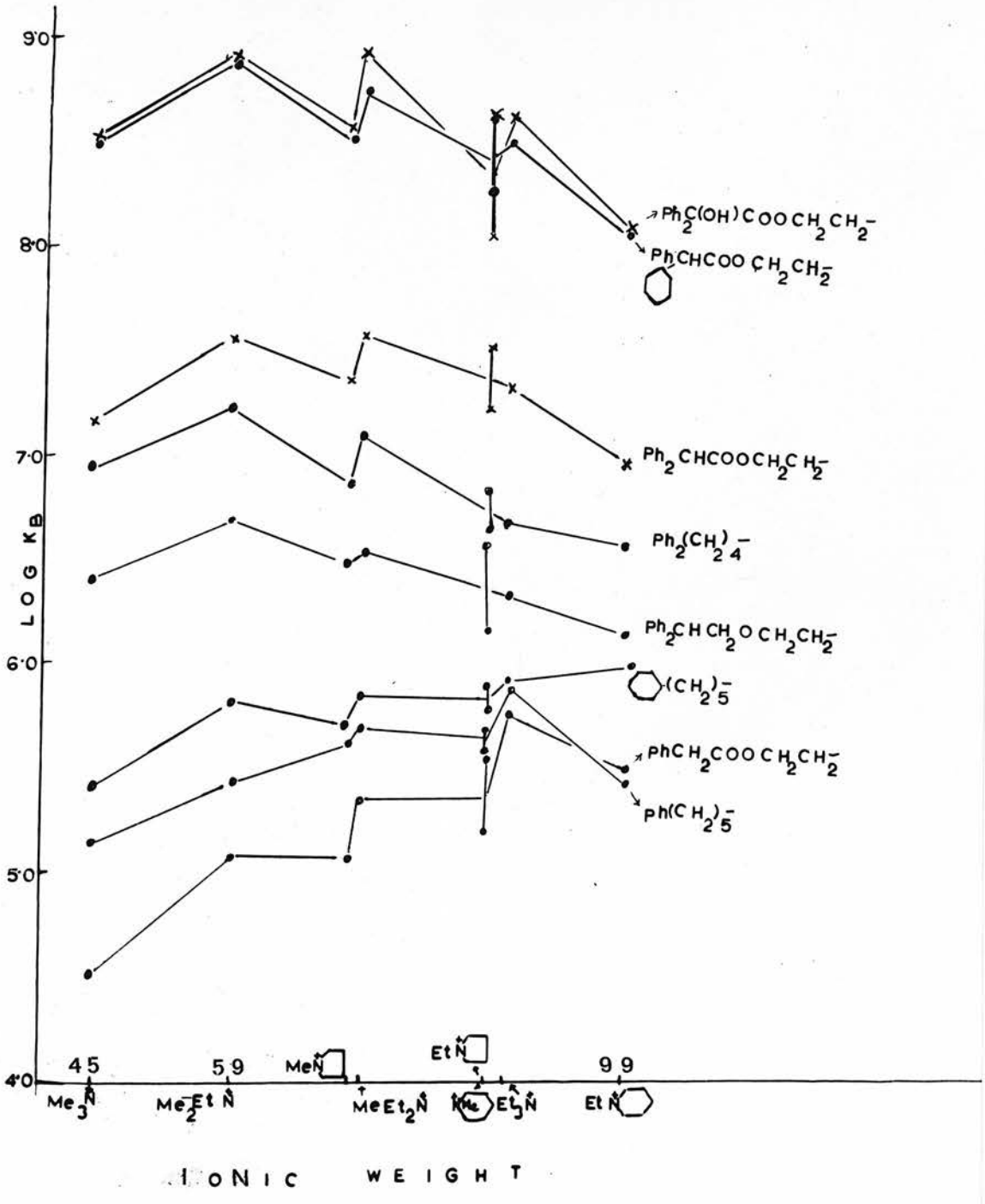


FIGURE V. Graph showing the relationship between log affinity and ionic weight of the groups attached to the quaternary nitrogen atom. "Lower analogues" show a progressive rise in affinity with increasing ionic weight whereas the "higher analogues" show a progressive decline of affinity.

Effect of constitution of onium group on affinity constant of the compounds $R'\overset{+}{N}R_3$.

The affinity is compared with that of the trimethyl-ammonium salt, $R'\overset{+}{N}Me_3$; -f indicates the difference in the free energy of adsorption; values in parentheses indicate 95% confidence limits with a variance of 0.00486. K is the affinity constant of $R\overset{+}{N}Me_3$ and Ka is that for the other compounds.

TABLE XXVA

PHENYLPENTYL SERIES

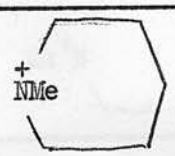
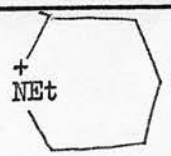
$\overset{+}{N}R_3$	log Ka/K	-f
$\overset{+}{N}Me_2Et$	(0.206) 0.267 (0.328)	(292) 378 (464)
$\overset{+}{N}MeEt_2$	(0.476) 0.535 (0.593)	(674) 758 (840)
$\overset{+}{N}Et_3$	(0.658) 0.715 (0.772)	(932) 1013 (1094)
	(0.384) 0.452 (0.521)	(544) 640 (738)
	(0.377) 0.452 (0.527)	(534) 640 (747)
	(0.436) 0.505 (0.574)	(618) 716 (813)
	(0.202) 0.277 (0.352)	(286) 393 (499)

TABLE XXV B

CYCLOHEXYL-PENTYL SERIES

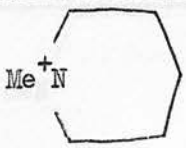
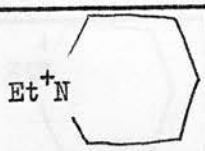
$\overset{+}{N}R_3$	Log $\frac{Ka}{K}$	-f
$\overset{+}{N}Me_2Et$	(0.336) 0.414 (0.492)	(476) 587 (697)
$\overset{+}{N}MeEt_2$	(0.360) 0.442 (0.524)	(510) 626 (743)
$\overset{+}{N}Et_3$	(0.435) 0.511 (0.587)	(616) 724 (832)
	(0.215) 0.291 (0.367)	(305) 412 (520)
	(0.321) 0.403 (0.485)	(455) 571 (687)
	(0.432) 0.508 (0.584)	(612) 720 (828)
	(0.532) 0.614 (0.696)	(754) 870 (986)

TABLE XV C

PHENYLACETOXYETHYL SERIES

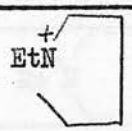
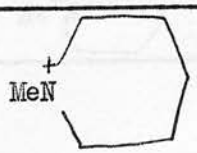
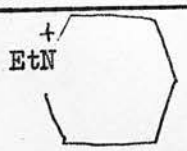
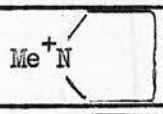
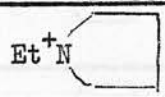
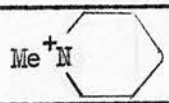
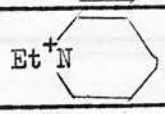
$\overset{+}{N}R_3$	Log $\frac{Ka}{K}$	-f
$\overset{+}{N}Me_2Et$	(0.493) 0.566 (0.638)	(699) 802 (904)
$\overset{+}{N}MeEt_2$	(0.747) 0.818 (0.888)	(1058) 1159 (1258)
$\overset{+}{N}Et_3$	(1.179) 1.252 (1.325)	(1671) 1774 (1878)
	(0.478) 0.551 (0.624)	(677) 781 (884)
	(0.964) 1.035 (1.106)	(1366) 1467 (1567)
	(0.588) 0.661 (0.734)	(833) 937 (1040)
	(0.921) 0.992 (1.063)	(1305) 1406 (1506)

TABLE XXV D

CYCLOHEXYLACETOXY-ETHYL SERIES

$\overset{+}{N}R$	Log $\frac{Ka}{K}$	-f
$\overset{+}{N}Me_2Et$	(0.456) 0.521 (0.586)	(646) 738 (830)
$\overset{+}{N}MeEt_2$	(0.481) 0.546 (0.611	(682) 774 (866)
$\overset{+}{N}Et_3$	(0.476) 0.541 (0.606)	(674) 767 (859)
	-	-
	-	-
	-	-
	-	-

TABLExXV E PHENYLCYCLOHEXYLACETOXYETHYL SERIES

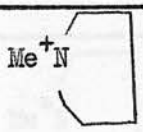
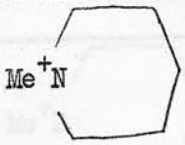
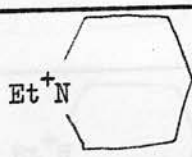
$\overset{+}{N}R_3$	Log $\frac{Ka}{K}$	-f
$\overset{+}{N}Me_2Et$	(0.315) 0.386 (0.457)	(446) 547 (648)
$\overset{+}{N}MeEt_2$	(0.194) 0.267 (0.339)	(275) 378 (480)
$\overset{+}{N}Et_3$	(-1.981) 0.052 (0.123)	(-27) 74 174
	(-1.997) 0.070 (0.142)	(-4) 99 (201)
	(0.070) 0.146 (0.222)	(99) 207 (315)
	(-1.730) -1.806 (-1.882)	(-383) -275 (-167)
	(-1.536) -1.607 (-1.678)	(-657) -557 (-456)

TABLE XV F

DIPHENYLPENTYL SERIES

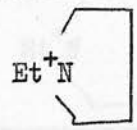
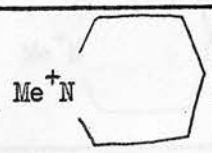
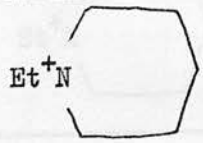
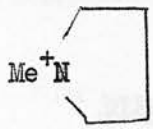
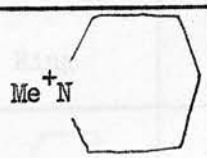
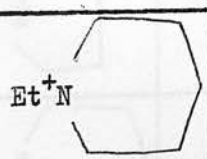
$^{+}NR_3$	Log $\frac{Ka}{K}$	-f
$^{+}NMe_2Et$	(0.277) 0.307 (0.387)	(393) 435 (548)
$^{+}NMeEt_2$	(0.078) 0.158 (0.238)	(111) 224 (337)
$^{+}NEt_3$	($\bar{1}$.682) $\bar{1}$.762 ($\bar{1}$.842)	(-450) -337 (-224)
	($\bar{1}$.862) $\bar{1}$.948 (0.342)	(-196) - 74 (48)
	($\bar{1}$.842) $\bar{1}$.902 ($\bar{1}$.978)	(-224) -139 (- 31)
	($\bar{1}$.640) $\bar{1}$.720 ($\bar{1}$.800)	(-510) -397 (-283)
	($\bar{1}$.574) $\bar{1}$.645 ($\bar{1}$.716)	(-604) -503 (-402)

TABLE XXV G

DIPHENYLETHOXYETHYL SERIES

$\overset{+}{N}R_3$	Log $\frac{Ka}{K}$	-f
$\overset{+}{N}Me_2Et$	(0.211) 0.280 (0.349)	(299) 397 (495)
$\overset{+}{N}MeEt_2$	(0.058) 0.127 (0.196)	(82) 170 (278)
$\overset{+}{N}Et_3$	($\bar{1}$.892) $\bar{1}$.961 (0.03)	(-153) (- 55) (43)
	(0.026) 0.095 (0.164)	(37) 135 (232)
	(0.107) 0.176 (0.245)	(152) 249 (347)
	($\bar{1}$.700) $\bar{1}$.769 ($\bar{1}$.838)	(-425) -327 (-229)
	($\bar{1}$.669) $\bar{1}$.738 ($\bar{1}$.807)	(-469) -371 (-273)

The effect on affinity of replacing N-methyl-pyrrolidinium (K) by N-ethyl-pyrrolidinium (Kx) and N-methyl piperidinium (K) by N-ethyl piperidinium (Kx). Values in parentheses indicate 95% confidence limits, with a variance of 0.00486.

TABLE XXVI A PHENYLPENTYL SERIES

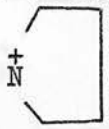
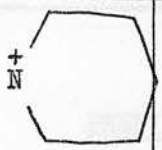
Ring	Log $\frac{Kx}{K}$	-f
	($\bar{1}$.917) $\bar{1}$.990 (0.813)	(-118) (-1) +115
	($\bar{1}$.735) (-1.813) ($\bar{1}$.895)	(-376) -265 (-149)

TABLE XXVI B DIPHENYLPENTYL SERIES

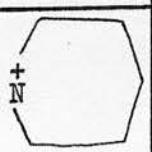
Ring	Log $\frac{Kx}{K}$	-f
	($\bar{1}$.866) -1.954 +.042	(-190) -65 (60)
	($\bar{1}$.847) -1.925 +.003	(-217) -106 +4

TABLE XXVI C

CYCLOHEXYL-PENTYL SERIES

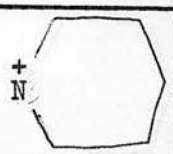
Ring	Log $\frac{Ka}{K}$	-f
	(0.038) 0.112 (.186)	(54) 159 (263)
	(0.032) 0.106 (0.180)	(45) 150 (255)

TABLE XXVI D

PHENYLACETOXYETHYL SERIES

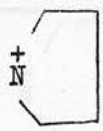
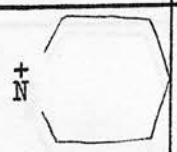
Ring	Log $\frac{Ka}{K}$	-f
	(0.278) 0.351 (0.423)	(394) 497 (599)
	(0.258) 0.331 (0.403)	(366) 469 (571)

TABLE XXVI E

CYCLOHEXYACETOXYETHYL SERIES

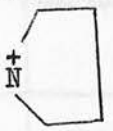
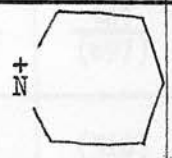
Ring	Log $\frac{Kx}{K}$	-f
	($\bar{1}$.999) 0.076 (0.152)	-0.6 107 (215)
	($\bar{1}$.726) $\bar{1}$.801 ($\bar{1}$.875)	(-388) -282 (-177)

TABLE XXVI F

DIPHENYLETHOXYETHYL SERIES

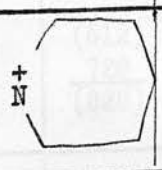
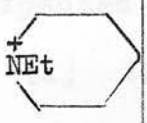
Ring	Log $\frac{Kx}{K}$	-f
	(-1.896) -1.969 (0.041)	(-147) - 44 (58)
	(0.012) 0.081 .149	(17) 115 (211)

TABLE XXVII

Summary of results: values of -f at 37°C accompanying changes in the onium group for the acetylcholine analogues. Parentheses contain 95% confidence limits.

$\overset{+}{N}R_3$	Phenyl-Pentyl Series	Cyclohexy-Pentyl Series	Phenyl-acet-oxy-ethyl Series	Cyclohexyl acetoxy-ethyl Series	Phenyl-cyclo-hexyl-acetoxy Series	Diphenyl Pentyl Series	Diphenyl ethyoxy-ethyl Series
$\overset{+}{N}Me_2Et$	(292) 378 (464)	(476) 587 (697)	(699) 802 (904)	(646) 778 (830)	(446) 547 (648)	(393) 435 (548)	(299) 397 (495)
$\overset{+}{N}MeEt_2$	(674) 758 (840)	(510) 626 (743)	(1058) 1159 (1258)	(682) 774 (866)	(275) 378 (480)	(111) 224 (337)	(82) 170 (278)
$\overset{+}{N}Et_3$	(937) 1013 (1094)	(616) 724 (832)	(1671) 1774 (1878)	(674) 767 (859)	(-27) 74 (174)	(-450) -337 (-224)	(-153) -55 (43)
	(544) 640 (738)	(305) 412 (520)	(677) 781 (884)		(-4) 99 (201)	(-196) - 74 (48)	(37) 135 (232)
	(534) 640 (747)	(455) 571 (687)	(1366) 1467 (1567)		(99) 207 (315)	(-224) -139 (-31)	(152) 249 (347)
	(618) 716 (813)	(612) 720 (828)	(833) 937 (1040)		(-383) (-275) (-167)	(-510) -397 (-283)	(-425) -327 (-229)
	(286) 393 (499)	(754) 870 (986)	(1305) 1406 (1506)		(-657) -557 (-456)	(-604) -503 (-402)	(-469) -371 (-273)

were also studied by Scott and the results of the first two compounds are not significantly different from those obtained in this work. The values for the methyldiethyl and triethyl ammonium compounds were, however, significantly different from Scott's results ($P < 0.001$; Table VIA) but the differences are not big.

In all the series of acetylcholine and analogues tested the replacement of one methyl group by an ethyl group increased the affinity 2-4 fold. This is in full agreement with the results of Ing, Dawes and Wajda (1945), Scott (1962) and Abramson (1964). The effect on affinity of further replacement of methyl groups by ethyl, however, is different in the different series of compounds. This indicates that the effect of changes in the composition of the onium groups is not really independent of the nature of the "body" of the molecule and raises two questions:

(1) Is the effect on affinity of making changes in the onium group related to the affinity of the compounds themselves, being greater in the molecules with lower affinity than those with the high affinity? This might be expected from Burgen's suggestions.

(2) Is the variation in the effect on affinity of replacing methyl by ethyl so great as to make it impossible to predict likely changes in the affinity of agonists?

Examination of Figures IV and IVA does not suggest

that changes in the composition of the onium groups affect molecules with lower affinity (lower analogues) more than with higher affinity (higher analogues). In TableXXVIIIthe affinity is compared with the biggest differences in affinity between the members of the same series. The biggest difference is with the phenyl-acetoxyethyl series, which has the lowest affinity, but with all the others the range is more or less the same even though the affinity varies 10,000 fold. Even with the compounds with the highest affinity the range is wide, 0.8 to 0.9, compared with 1.2 for the phenyl-acetoxyethyl series. The results, therefore, do not indicate a relation between the affinity and the change in affinity brought about by altering the onium group. It is, however, noticeable that the position of the compounds with highest and lowest affinity alters considerably in the series, depending on the affinity. The triethyl and ethylpiperidinium compounds, for example, have much higher affinity in the series with lower affinity than in those with high affinity.

In Figure V[illegible] there is a suggestion that in the "lower analogues" an increase in size is associated with increased affinity, whereas in the "higher analogues" an increase in size, beyond the replacement of one methyl group by ethyl, usually causes a decrease in affinity. The variation in affinity with composition of the onium groups seems to depend on whether there is

TABLE XXVIII

Differences between the highest and lowest values of log. affinity constants within the series.

Series	Log. K_B for $+NMe_3$ Comp.	Log. difference
Ph acetoxyethyl	4.53	1.252
Ph pentyl	5.18	0.715
Cyclohexyl Pentyl	5.41	0.614
Ph_2ethoxyethyl	6.41	0.542
Ph_2pentyl	6.95	0.545
Ph_2acetoxyethyl	7.16	0.574
Ph Cyclohexyl acetoxyethyl	8.50	0.779
Benziloyloxyethyl	8.51	0.868

one ring (phenyl or cyclohexyl) at the end of the molecule or two, i.e. there appears to be a pattern which is common to the "lower analogues" and a rather different pattern for the "higher analogues". It seems therefore that the binding of the onium group will be considerably affected by the binding of these rings which contribute quite substantially to the affinity of the whole molecule (see below).

The second question, the validity of using the estimates of the affinity of the antagonists to predict the relative affinities of agonists, must remain incompletely answered. However, in nine series of the antagonists with different affinities, replacement of one methyl group by an ethyl leads to an increase in affinity of 2-4 fold and the binding of agonists must be quite different if the same is not true with them. In fact, Stephenson (unpublished) has found that the affinity of pentylethyldimethyl ammonium is certainly not less than that for pentyltrimethyl ammonium though it may not be as much as twice. The graph for the affinity of the pentyl compounds (FigureIV) is not markedly different from that of the phenylacetoxyethyl compounds.

The size of the changes in affinity with the composition of the onium group indicates a change in free energy of 0.4 - 0.8 Kcal/per methylene group and this suggests changes in binding brought about by Van der

Waals and/or hydrophobic forces. Because of extreme dependence of Van der Waals forces on the close proximity of the interacting groups, it would seem more possible that hydrophobic forces are involved, particularly with "lower analogues" with which affinity rises with size. When the change in composition reduces the affinity, it must be assumed that the parts of the molecule which were formerly contributing to the binding can no longer do so, or do so less efficiently. Again the magnitude of the change in free energy is consistent with the idea that the bonds involved are Van der Waals or hydrophobic in nature. In some instances, e.g. with the isomeric methylpiperidinium and ethylpyrrolidinium compounds, it is clear that the shape of the onium groups is important, which might indicate that Van der Waals bonding is involved rather than hydrophobic bonds. With the higher compounds the flat pyrrolidinium compounds bind better than the piperidinium compounds. Only with the pentyl series the position is reversed, possibly with these the contributions from the hydrophobic forces are more important than those from Van der Waals forces.

The effects of changes of the constitution of the body on the adsorbability are shown in Table XXIX & XXX.

The substitution of one phenyl or cyclohexyl ring at the end of the molecule increased the affinity between 2.5 - 63 fold compared with that of compounds in the

Effect of constitution of the group R' (body) on affinity constant of the compounds $R'NR_3$. The affinity constant of a compound is compared with that of the member of the phenylpentyl series with the same onium group, -f indicates the difference in the free energy of adsorption; values in parentheses indicate 95% confidence limits, with a variance of 0.00486.

TABLE XXIXA

TRIMETHYL AMMONIUM COMPOUND

Body	Log $\frac{KC}{K}$	-f
Ph_2-Pentyl	(1.704) 1.771 (1.837)	(2415) 2509 (2603)
Cyclohexyl-pentyl	(0.157) 0.232 (0.306)	(222) 329 (434)
Phenylacetoxy-ethyl	($\bar{1}$.287) -1.354 (-1.421)	(-1010) -915 (-820)
Cyclohexyl-acetoxyethyl	($\bar{1}$.727) -1.786 (-1.845)	(-387) -303 (-219)
Ph-cyclohexyl-acetoxyethyl	(3.258) 3.325 (3.391)	(4616) (4711) (4805)
Ph_2ethoxy-ethyl	(0.901) 0.964 (1.027)	(1277) 1366 (1455)

TABLE XXIXB

ETHYLDIMETHYL AMMONIUM COMPOUND

Body	Log $\frac{KC}{K}$	-f
Ph_2Pentyl	(1.727) 1.811 (1.895)	(2447) 2566 (2685)
Cyclohexyl-pentyl	(0.300) 0.379 (0.457)	(425) 537 (648)
Ph-acetoxy-ethyl	($\bar{1}$.574) -1.653 ($\bar{1}$.731)	(-603) -492 (-381)
Cyclohexyl acetoxyethyl	($\bar{1}$.963) 0.040 (0.116)	(-52) 57 (164)
Ph-cyclohexyl-acetoxy ethyl	(3.367) 3.444 (3.520)	(4771) 4880 (4988)
Ph_2 ethoxy-ethyl	(1,170) 1.247 (1.323)	(1658) 1767 (1875)

TABLE XXIXC

METHYLDIETHYL AMMONIUM COMPOUND

Body	Log $\frac{K_c}{K}$	(-f)
Ph_2-Pentyl	(1.319) 1.394 1.468	(1869) 1975 (2080)
Cyclohexyl-pentyl	(0.070) 0.139 (0.208)	(99) 197 (295)
Ph-acetoxy-ethyl	(-0.427) -0.363 (-0.298)	(-605) -514 (-422)
Cyclohexyl-acetoxy-ethyl	(-0.268) -0.203 (-0.138)	(-370) -288 (-196)
Ph-cyclohexyl-acetoxy-ethyl	(2.990) 3.057 (3.124)	(4237) 4332 (4427)
Ph_2-ethoxy-ethyl	(0.761) 0.826 (0.891)	(1078) 1170 (1263)

TABLE XXIXD

TRIETHYL AMMONIUM COMPOUND

Body	Log $\frac{K_c}{K}$	(-f)
Ph_2-Pentyl	(0.747) 0.818 (0.888)	(1058) 1159 (1252)
Cyclohexyl- Pentyl	(-0.033) 0.028 (0.089)	(-47) 39 (126)
Ph-acetoxy- Ethyl	(-0.172) -0.109 (-0.046)	(-244) -154 (-65)
Cyclohexyl- Acetoxy- Ethyl	(-0.366) -0.305 (-0.244)	(-519) -432 (-346)
Ph-cyclohexyl- Acetoxy- Ethyl	(2.612) 2.672 (2.733)	(3701) 3786 (3873)
Ph_2-Ethoxy- Ethyl	(0.417) 0.480 (0.543)	(591) 680 (769)

TABLE XXIXE. METHYL PYRROLIDINIUM COMPOUND

Body	Log $\frac{K_c}{K}$	-f
Ph_2-Pentyl	(1.178) 1.266 (1.354)	(1669) (1794) (1918)
Cyclohexyl-Pentyl	(-0.003) 0.070 (0.142)	(-4) 99 (201)
Ph-acetyoxy-Ethyl	(-0.624) -0.548 (-0.471)	(-884) -776 (-667
Ph-cyclohexyl-acetoxy-ethyl	(2.865) 2.942 (3.018)	(4060) 4169 (4276)
Ph_2-Ethoxy-ethyl	(0.803) 0.876 .948	(1138) 1241 (1343)

TABLEXXIXF ETHYL PYRROLIDINIUM COMPOUND

Body	$\text{Log } \frac{K_c}{K}$	-f
Ph_2- Pentyl	(1.139) 1.221 (1.303)	(1614) 1730 (1846)
Cyclohexyl-Pentyl	(0.101) 0.183 (0.265)	(143) 259 (375)
Ph-acetoxy-ethyl	(-0.139) -0.063 (+0.013)	(-197) - 89 (18)
Ph-cyclohexyl-acetoxy-ethyl	(2.988) 3.070 (3.152)	(4234) 4350 (4466)
Ph_2-ethoxy-ethyl	(0.881) 0.958 (1.034)	(1248) 1357 (1465)

TABLE XXIXG METHYL PIPERIDINIUM COMPOUND

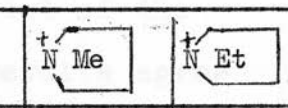
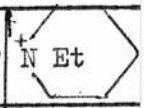
Body	Log $\frac{K_c}{K}$	-f
Ph_2- Pentyl	(0.904) 0.986 (1.068)	(1281) 1397 (1513)
Cyclohexyl- Pentyl	(0.160) 0.235 (0.309)	(227) 333 (438)
Ph-acetoxy- ethyl	(-0.566) -0.490 (-0.413)	(-802) -694 (-585)
Ph-cyclohexyl- acetoxy- ethyl	(2.548) 2.626 (2.704)	(3610) 3721 (3831)
Ph_2-ethoxy- ethyl	(0.421) 0.498 (0.574	(596) 706 (813)

TABLE XXIXH ETHYL PIPERIDINIUM COMPOUND

Body	Log $\frac{K_c}{K}$	-f
Ph_2- Pentyl	(1.062) 1.139 (1.215)	(1505) 1614 (1722)
Cyclohexyl- Pentyl	(0.487) 0.569 (0.651)	(690) 806 (922)
Ph-acetoxy- ethyl	(-0.007) 0.069 (0.145)	(-10) 98 (205)
Ph-cyclohexyl- acetoxy- ethyl	(2.578) 2.655 (2.731)	(3653) 3762 (3870)
Ph_2ethoxy- ethyl	(0.618) 0.695 (0.771)	(876) 985 (1092)

TABLE XXX

Summary of the results: values of -f cal/mole at 37°C accompanying changes in the body of the molecule of the acetylcholine analogues; values in parentheses indicate 95% confidence limits; compounds of Phenylpentyl series taken as standard.

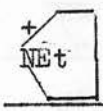
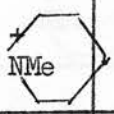
Body	$\overset{+}{N}Me_3$	$\overset{+}{N}Me_2Et$	$\overset{+}{N}MeEt_2$	$\overset{+}{N}Et_3$				
Cyclohexyl-Pentyl	(222) 329 (434)	(425) 537 (648)	(99) 197 (295)	(-47) 39 (126)	(-4) 99 (201)	(143) 259 (375)	(227) 333 (438)	(690) 806 (922)
Ph-acetoxy ethyl	(-1010) (-915) (-820)	(-603) -492 (-381)	(-605) -514 (-422)	(-244) -154 (-65)	(-884) -776 (-667)	(-197) -89 (18)	(-802) -694 (-585)	(-10) 98 (205)
Cyclohexyl-acetoxy-ethyl	(-387) -303 (-219)	(-52) 57 (164)	(-370) -288 (-196)	(-519) -432 (-346)	-	-	-	-
Ph_2-Pentyl	(2415) 2509 (2603)	(2447) 2566 (2685)	(1867) 1975 (2080)	(1058) 1159 (1252)	(1669) 1794 (1918)	(1614) 1730 (1846)	(1281) 1397 (1513)	(1505) 1614 (1722)
Ph-Cyclohexyl acetoxy-ethyl	(4616) 4711 (4805)	(4771) 4880 (4988)	(4237) 4332 (4427)	(3701) 3786 (3873)	(4060) 4169 (4276)	(4234) 4350 (4466)	(3610) 3721 (3831)	(3653) 3762 (3870)
Ph_2ethoxy-ethyl	(1277) 1366 (1455)	(1658) 1767 (1875)	(1078) 1170 (1263)	(591) 680 (769)	(1138) 1241 (1343)	(1248) 1357 (1465)	(596) 706 (813)	(876) 985 (1092)

n-pentyl series, the substitution of two phenyl rings increased the affinity between 25 - 3162 fold and with the substitution of one phenyl and one cyclohexyl ring together in the compounds (with the ester link) the affinity rose between 1995 - 79430 times that of the corresponding n-pentyl compound. These results agree with the observations of Meier and Hoffmann (1941), Cunningham (1949), Lands (1951) and Lands et al. (1956).

In terms of the free energy of adsorption, the increase for a single phenyl ring is 1.2 - 2.0 Kcal/mole, that for a cyclohexyl ring is 1.5 - 2.6 Kcal/mole and the energy for two phenyl rings is 2.6 - 4.6 Kcal/mole. The effects, however, are different in different series (TableXXXI). For example, in the series with a straight methylene chain, the introduction of the second phenyl ring has much the same effect as the introduction of the first. When the esters are compared, however, the introduction of the second phenyl ring has a much bigger effect on binding (about 3 Kcal/mole as opposed to 2 Kcal/mole). The effect is even greater when a phenyl ring is introduced into the cyclohexyl esters, when the binding is increased by between 4 and 5 Kcal/mole. In both the pentyl and the acetoxyethyl series the cyclohexyl compounds have a higher affinity than the phenyl ones. It would be interesting to have accurate information about the dicyclohexyl compounds. Some of these compounds have been tested by Meier and

TABLE XXXIA

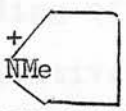
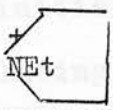
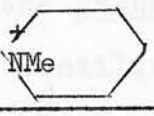
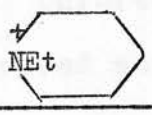
The effects of Benzene ring in the "body" on free energy of adsorption (-f). The figures were obtained by comparison of log affinity constants of the corresponding compounds in the various series.

	1	2	3	4	5	6
$+NR_3$	Log affinity of Pentyl TMA	$\frac{Ph(CH_2)_5}{\text{Pentyl TMA}}$	$\frac{Ph_2CH(CH_2)_4}{Ph(CH_2)_5}$	$\frac{Ph_2CH\ COO(CH_2)_2}{Ph\ CH_2COO(CH_2)_2}$	Ratios between Column 2 and 3	Ratios between Column 2 and 4
$+NMe_3$	3.733 (Agonist)	2049	2509	3724	1.2	1.8
$+NMe_2Et$	3.970 (Agonist)	2091	2566	3520	1.2	1.7
$+NMeEt_2$	4.399	1863	1975	3174	1.06	1.7
$+NEt_3$	4.589	1949	1159	2245	0.6	1.15
	4.166 (Partial Agonist)	2077	1794	3337	0.86	1.6
	4.370	1787	1730	2821	0.97	1.58
	4.815	1231	1397	2935	1.13	2.38
	4.576	1289	1614	2114	1.25	1.64

NB. Results for the compounds of n-pentyl series were obtained by R.P. Stephenson. (Unpublished).

TABLE XXXIB

The effects of cyclohexyl ring in the "body" on the free energy of absorption (-f). Figures were obtained by comparison of the log affinity of the corresponding compounds of the series.

$^{+}NR_3$	Cyclohexyl Pentyl Pentyl TMA	Ph-Cyclohex-acetoxyethyl Ph-acetoxyethyl
$^{+}NMe_3$	2378	5627
$^{+}NMe_2Et$	2628	5372
$^{+}NMeEt_2$	2060	4846
$^{+}NEt_3$	1889	3941
	2177	4945
	2046	7367
	1564	4415
	2094	3664

Hoffmann (1941) and Levy and Tochoubar (1947) but it is impossible to make an estimate of their affinity constants from their (rather conflicting) observations.

The above results can equally be set out to show that the effect of the ester group depends on the number of phenyl or cyclohexyl rings (Table XXXII). In the "lower analogues" with only one big substituent, the ester group invariably reduces the affinity compared with the methylene analogues. In the "higher analogues", however, the ester group appreciably increases affinity. The ether group, on the other hand, lowers affinity, even in the higher analogues.

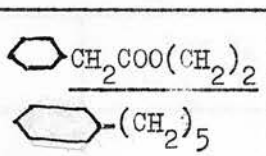
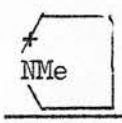
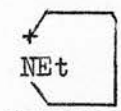
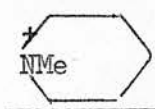
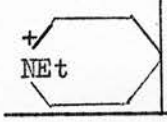
Tropines:

With the tropines it is again clear that the substitution on one end of the molecule influences the binding of the other end (Table XXXIII). The simple unesterified derivatives of tropine all have the same rather weak affinities. With the esters, however, the effect of replacing a methyl group by ethyl is to reduce affinity, in the pseudotropines more than with the tropines and in the benzilic esters more than with diphenylacetyl esters.

Unfortunately, the monophenyl esters have not been prepared and the only comparison which can therefore be made is that which should show the effect of the hydroxyl group on affinity (TableXXXIV). The values fall within the range 1.7 - 2.2 Kcal which is the same as the difference between the benziloyl and diphenylacetyl compounds studied by Scott and later by Abramson. This

TABLE XXXII

The effect of ester and ether oxygen groups in the body on the free energy of adsorption (-f). The figures were obtained by comparison of the log affinity of the corresponding compounds of the series.

	Ester Group (-CO-O)			Ether Group (-O-)
$\overset{+}{N}R_3$	$\frac{Ph\ CH_2COO(CH_2)_2}{Ph\ (CH_2)_5}$		$\frac{Ph_2CH\ COO(CH_2)_2}{Ph_2CH\ (CH_2)_4}$	$\frac{Ph_2CH\ CH_2O(CH_2)_2}{Ph_2CH\ (CH_2)_4}$
$\overset{+}{N}Me_3$	-915	-632	299	-761
$\overset{+}{N}Me_2Et$	-492	-480	462	-799
$\overset{+}{N}MeEt_2$	-514	-485	684	-805
$\overset{+}{N}Et_3$	-154	-472	931	-479
	-776	-	767	-553
	-89	-	1002	-373
	-694	-	843	-691
	98	-	598	-629

NB. Results for Ph_2 CH COO $(CH_2)_2$ were obtained by Abramson (1964).

TABLE XXXIII SUMMARY OF RESULTS: RATIOS OF LOG K_B AND VALUES OF (-f) OF THE CHANGES IN THE ALKYL-RADICAL FROM ETHIODIDE (K) TO METHIODIDE (K_a); THE VALUES IN THE PARENTHESES INDICATE 95% CONFIDENCE LIMITS, WITH A VARIANCE OF 0.00681

"Body"	Log $\frac{K_a}{K}$	(-f)
Benziloyl-tropine	(1.269) 1.343 (1.417)	(1798) 1903 (2008)
Benziloyl-Pseudo tropine	(1.643) 1.731 (1.819)	(2328) 2453 (2577)
Diphenyl-acetyl tropine	(0.711) 0.805 (0.899)	(1007) 1141 (1274)
Diphenylacetyl-Pseudo-tropine	(1.242) 1.356 (1.470)	(1760) 1921 2083
Tropine	(-0.009) 0.004 (0.108)	(-140) 6 (153)
Pseudo-tropine	(-0.098) 0.000 (0.098)	(-138) 0.00 (138)

TABLE XXXIV EFFECT ON AFFINITY AND (-f) OF REPLACING DIPHENYLACETYL GROUP (K) BY BENZILOYL GROUP (K_a).

VALUES IN THE PARENTHESES INDICATE 95% CONFIDENCE LIMITS

	Tropine		Pseudo Tropine	
	Meth.	Eth.	Meth.	Eth.
$\text{Log } \frac{K_a}{K}$	(1.690) 1.774 (1.858)	(1.150) 1.236 (1.322)	(1.474) 1.588 (1.702)	(1.121) 1.213 (1.305)
-f	(2395) 2514 (2633)	(1630) 1751 (1873)	(2089) 2250 (2412)	(1588) 1719 (1849)

is consistent with the hydrogen bonding involving the hydroxyl group. The effect is the same in both tropines and pseudotropines but is smaller with the ethiodides than with the methiodides (Table XXXV).

Conclusions

The results obtained in the study of these antagonists show that the assumptions of Barlow, Scott and Stephenson (1963), that affinity is made up of components which are additive, is far too simple. An extreme example is the effect of replacing esters (-CO-O-) by ethylene ($-CH_2CH_2-$); in the monophenyl series this increases affinity whereas in the diphenyl series it decreases affinity. On the other hand, there is no obvious relationship between the effects of substituents on affinity and the affinity itself. It appears, rather, that effects of changes in structure on affinity are related to the chemical nature of the compounds and within series, for example, within the "lower analogues" or within the "higher analogues", the effects do follow a regular pattern, indicating that the binding is made up of components which are additive. The differences between the series, however, indicate that different types of molecule bind in different ways and even within series it appears that there are slight differences in the ways in which the individual compounds become bound.

TABLE XXXV

EFFECTS ON AFFINITY AND (-f) OF THE ARRANGEMENT OF THE 3-HYDROXYL GROUP OF THE TROPINE RING; THE RATIO IS OF, K_a, FOR TROPINE DERIVATIVE RELATIVE TO K, FOR THE PSEUDO TROPINE DERIVATIVES

Body		Meth.	Eth.
Benziloyl-tropine	Log $\frac{K_a}{K}$	(0.546) $\frac{0.624}{(0.702)}$	(0.932) $\frac{1.010}{(1.088)}$
	-f	(774) $\frac{884}{(995)}$	(1321) $\frac{1431}{(1542)}$
Diphenyl-acetyl tropine	Log $\frac{K_a}{K}$	(0.323) $\frac{0.439}{(0.555)}$	(0.895) $\frac{0.989}{(1.083)}$
	-f	(458) $\frac{662}{(780)}$	(1268) $\frac{1401}{(1535)}$

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