THE KINETICS OF ACTION OF ACETYLCHOLINE ANTAGONISTS ON GUINEA-PIG ILEUM

F. Roberts

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University of Edinburgh

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SUMMARY

Several workers, e.g., Paton (1961) and Paton & Rang (1965) have used the observed rates of onset and offset of antagonism on isolated guinea-pig ileum to calculate the antagonist-receptor association and dissociation rate constants, $k_1$ and $k_2$. This involves the assumption that the interaction of the drug with the receptors is rate limiting rather than the access of the drug to the receptors.

The kinetic behaviour of three antagonists on guinea-pig ileum was investigated and compared with the predictions of the interaction limited model. Intact pieces of guinea-pig ileum were suspended in Tyrode's solution and the contractions produced by carbachol were recorded isotonically.

The kinetic behaviour of the very slow antagonist benziloyl tropine methyl iodide (BTrMe) was examined in three types of experiments:

1. Onset and recovery from various concentrations of the antagonist were followed.

2. The decrease in occupancy of BTrMe, produced when a concentration of the 'fast' antagonist pentyl triethylammonium iodide (pentyl TEA) was superimposed, was also followed.

3. The interaction between BTrMe and pentyl TEA was also examined in experiments in which the concentration of BTrMe was adjusted so that its occupancy in equilibrium with the pentyl TEA was the same as that in equilibrium in the absence of pentyl TEA.

The rate of onset and recovery from pentyl TEA or lachesine were also investigated, lachesine being intermediate in speed between pentyl TEA and BTrMe. The rate of offset of lachesine on superimposition of pentyl TEA, or alternatively octyl TMA or $\text{Ph}_2\text{AOEMe}_2\text{Et}$, was also followed.
The kinetic behaviour of BTrMe, lachesine & pentyl TEA was not found to be consistent with the predictions of the interaction limited model and therefore it was concluded that some sort of access limitation must be involved.

As the rates of onset and offset, when an antagonist is added or removed from the bathing solution, appear to be access limited, values of $k_1$ and $k_2$ cannot be determined from such kinetic measurements. However the rate of offset of a slow antagonist on superimposition of a high concentration of a fast antagonist may be limited by the rate at which it dissociates from the receptors, but the possibility can not be ruled out that a different access limitation is then operating.

Experiments were also carried out using different experimental methods; longitudinal muscle strips or intact pieces of ileum were used, an isometric transducer or an isotonic lever, Krebs solution or Tyrode's solution and pentyl TMA or carbachol. The results of these experiments indicate that the discrepancies between the kinetic behaviour of these antagonists and the interaction limited model are unlikely to be due to such differences in experimental method.
## CONTENTS

<table>
<thead>
<tr>
<th>Summary</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Contents</td>
<td>4</td>
</tr>
<tr>
<td>Notation</td>
<td>8</td>
</tr>
</tbody>
</table>

### INTRODUCTION

1) The relationship between the structure of a drug and its activity
2) The onset and recovery from antagonism - theory
3) A note on the procedure used to follow antagonism
4) The rates of onset and offset of antagonists on guinea-pig ileum
5) The interaction between fast-acting and slow-acting antagonists on guinea-pig ileum
6) The kinetics of tetrodotoxin's action
7) Observations that are difficult to explain if interaction is rate-limiting - muscarinic antagonists on guinea-pig ileum
8) A note on the rate of dissociation of an antagonist when an agonist is superimposed
9) The kinetics of tetrodotoxin - an observation that suggests that access may be rate-limiting
10) The kinetics of antagonists on other isolated tissues
11) The lack of an 'appropriate' access limited model
12) Simply diffusion - theory.
13) Could the kinetics of antagonists be diffusion limited?
14) Could the kinetics of agonists be diffusion limited?
15) The general relationship between the potency of a drug and the rate at which it acts
16) The biophase model - theory
17) Is there a barrier to diffusion?  40
18) The possible involvement of a barrier in adrenergic systems  42
19) The concentration of a drug to which the receptors are  44
   exposed
20) The biophase model - summary  47
21) The limited biophase model  48
22) The chief argument against the limited biophase model  51
23) Summary of the introduction  53

INVESTIGATIONS OF THE KINETICS OF CERTAIN COMPETITIVE ANTAGONISTS
ON GUINEA-PIG ILEUM

METHODS  55

PART I  THE KINETICS OF BTrMe  61
I.1 The kinetics of onset and offset of BTrMe  63
I.2 The interaction between BTrMe and pentyl TEA - the decrease  70
   in BTrMe's occupancy following the superimposition of
   pentyl TEA
I.3 The interaction between BTrMe and pentyl TEA when the  79
   concentration of BTrMe is adjusted to maintain its
   equilibrium occupancy
I.4 An examination of the assumptions used in Part I  85
  i) The relationship between BTrMe's occupancy and the apparent  85
dose ratio
  ii) The equilibrium dose ratio produced by various concentrations  90
      of BTrMe
  iii) The dose ratio produced by pentyl TEA alone and together  92
       with BTrMe
  iv) The rate of action of pentyl TEA  93

SUMMARY OF PART I  96
## PART II THE KINETICS OF LACHESINE

### II.1 The kinetics of onset and offset of lachesine

### II.2 The rate of offset of lachesine on superimposition of pentyl TEA

### SUMMARY OF PART II

### DISCUSSION

## PART III

### III.1 The kinetics of BTrMe using muscle strips and an isometric transducer

### III.2 The kinetics of BTrMe using the agonist pentyl TMA

### III.3 The kinetics of lachesine using muscle strips or intact pieces of ileum, and pentyl TMA or carbachol

### III.4 The kinetics of lachesine using intact pieces of ileum or muscle strips, and an isotonic lever or an isometric transducer

### III.5 The kinetics of lachesine when the preparation used is bathed in Krebs solution rather than Tyrode's

### DISCUSSION OF SECTIONS III.1-5

1. The discrepancies found in Parts I and II between the kinetics of lachesine and BTrMe and the predictions of the interaction limited model

2. The effect of agonist, lever, preparation and bathing solution on the kinetics of antagonism

3. The discrepancies between the kinetic observations made by different groups of workers

### III.6 The relationship between the limiting value of the rate of offset and $k_2$

### III.7 The kinetics of BTrMe following treatment of the tissue with an irreversible antagonist

### III.8 The kinetics of lachesine using hexyl TMA
DISCUSSION - ACCESS LIMITED MODELS
The role of receptor binding
The kinetics of antagonists on longitudinal muscle strips or intact pieces of ileum
The relationship between the structure and affinity of a drug, its concentration and the observed rate of antagonism
CONCLUSION
APPENDIX
The use of different agonists in antagonist affinity constant estimations
The increase in the slope of the log dose-response curve in the presence of Ph₂AOEMe₂Et
REFERENCES
NOTATION

The following system of symbols, with subscripts when appropriate, has been used:

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>The concentration of an agonist in the absence of antagonist(s)</td>
<td>M</td>
</tr>
<tr>
<td>A</td>
<td>The concentration of an agonist in the presence of antagonist(s)</td>
<td>M</td>
</tr>
<tr>
<td>B</td>
<td>The concentration of an antagonist</td>
<td>M</td>
</tr>
<tr>
<td>c</td>
<td>The concentration of a drug</td>
<td>M</td>
</tr>
<tr>
<td>d</td>
<td>The concentration of a drug = c x K_{aff}</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>The diffusion coefficient of a drug</td>
<td>cm² sec⁻¹</td>
</tr>
<tr>
<td>DR</td>
<td>Dose Ratio = A/a</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>The concentration of a fast-acting antagonist</td>
<td>M</td>
</tr>
<tr>
<td>k₁</td>
<td>Drug-receptor association rate constant</td>
<td>sec⁻¹ M⁻¹</td>
</tr>
<tr>
<td>k₂</td>
<td>Drug-receptor dissociation rate constant</td>
<td>sec⁻¹</td>
</tr>
<tr>
<td>k_{in}</td>
<td>The rate constant for the rate of entry into the biophase</td>
<td>sec⁻¹</td>
</tr>
<tr>
<td>k_{out}</td>
<td>The rate constant for the rate at which a drug leaves the biophase</td>
<td>sec⁻¹</td>
</tr>
<tr>
<td>K_{aff}</td>
<td>The affinity constant of a drug</td>
<td>M⁻¹</td>
</tr>
<tr>
<td>K</td>
<td>The affinity constant of a drug</td>
<td>M⁻¹</td>
</tr>
<tr>
<td>l</td>
<td>distance</td>
<td>cm</td>
</tr>
<tr>
<td>M</td>
<td>The binding capacity of the receptors - moles/unit wet weight of tissue</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>Number of estimations made</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>Occupancy - the proportion of the receptors occupied by a drug</td>
<td></td>
</tr>
<tr>
<td>Q</td>
<td>The rate of transfer of a diffusing substance</td>
<td>moles cm⁻² sec⁻¹</td>
</tr>
<tr>
<td>t</td>
<td>Time or</td>
<td>secs</td>
</tr>
<tr>
<td></td>
<td>The time constant for an exponential increase or decrease in occupancy</td>
<td>mins or secs</td>
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contd/
\[ T = t \times k_{out} \]

\[ x \quad \text{Distance} \quad \text{cm} \]

\[ V \quad \text{Biophase Volume} \quad \text{litres/unit wet weight of tissue} \]

In addition the following are used as subscripts:

- \( \circ \) - at zero time
- \( t \) - at time \( t \)
- \( \infty \) - at affinity, i.e. when the drug(s) are at equilibrium with the receptors
- \( a \) - when referring to the concentration of a drug in the aqueous phase, i.e. in the bathing solution
- \( A \) - when referring to an agonist
- \( b \) - when referring to the concentration of a drug in the biophase, i.e. in the proximity of the receptors
- \( B \) - when referring to an antagonist
- \( F \) - when referring to a fast-acting antagonist
- \( F+SL \) - when a fast and slow antagonist are acting together
- \( i \) - when referring to the concentration of a drug in the \( i^{th} \) compartment
- \( \text{on} \) - when referring to the onset of antagonism
- \( \text{off} \) - when referring to the offset of antagonism
- \( SL \) - when referring to a slow-acting antagonist
1) The relationship between the structure of a drug and its activity

There are many ways in which the relationship between a drug and its receptor can be investigated. These include attempts to isolate receptors, e.g. Ehrenpreis (1960), Miledi, Molinoff & Potter (1970), to characterize receptors using various reagents, e.g. Schild (1960), the use of model systems, e.g. Danielli & Davson (1935), and the use of mathematical models, e.g. Clark (1937), Paton (1961) and Karlin (1967).

One of the most successful approaches has been to study the relationship between the structure of a drug and its activity. Such structure activity relationships assume that for the formation of a drug receptor complex there has to be a measure of structural complementarity between the drug and the receptor. Competitive antagonists have been particularly important in these studies because the degree of antagonism they produce is considered to depend only on their affinity for the receptors, whereas the activity of agonists depends on their affinity and also their efficacy. (Throughout this study Stephenson's (1956) model of drug action has been used for convenience and because, as will be shown, the kinetics of antagonists appear to be access limited to a significant extent and therefore would not be expected to discriminate between this model and that of Paton (1961) or that of Karlin (1967).)

The affinity constant of a competitive antagonist can be determined on the following basis:

Both antagonists and agonists are assumed to combine with the receptors according to the law of mass action in the following way:
\[
\text{DRUG} + \text{RECEPTOR} \xrightarrow{k_1} \text{DRUG-RECEPTOR COMPLEX} \xrightarrow{k_2}
\]

where \( k_1 \) and \( k_2 \) are the drug-receptor association and
dissociation constants respectively, and the ratio \( k_1/k_2 \)
is the drug-receptor affinity constant denoted by \( K_{\text{aff}} \).

Therefore if a concentration, \( a \), of a drug whose affinity
constant is \( K_A \) equilibrates with the receptors, the
proportion of the receptors that this drug will occupy at
equilibrium, \( p_A \), is related to \( a \) and \( K_A \) thus:
\[
p_A = \frac{aK_A}{1 + aK_A}
\]

However if another drug affinity constant \( K_B \) is also present
in a concentration \( B \), then the proportion of receptors
occupied by the first drug, as shown by Gaddum (1937), will be
\[
\frac{aK_A}{1 + aK_A + BK_B}
\]

Then if it is assumed that if a concentration, \( a \), of agonist
produces a response \( r \) in the absence of antagonist, whereas
a concentration \( A \) is required to produce the same response in
the presence of a concentration \( B \) of antagonist:
\[
\frac{aK_A}{1 + aK_A} = \frac{AK_A}{1 + AK_A + BK_B}
\]

This rearranges to give:
\[
A = \frac{1 + BK_B}{a}
\]

(Schild, 1949)

where \( K_A \) is the affinity constant of the agonist, \( K_B \) is the
affinity constant of the antagonist, and the ratio \( A/a \) is
called the dose ratio and is denoted \( \text{DR} \).
On this basis the affinity constants of many competitive antagonists have been estimated and the relationship between affinity and chemical structure observed. However the affinity constant is the ratio of the two constants $k_1$ and $k_2$. Therefore antagonists with the same affinity do not necessarily have the same association and dissociation rate constants. Conversely it is not possible to determine how much the change in affinity produced by a certain structural change is due to a change in $k_1$ and how much to $k_2$.

In addition as Burgen (1965) pointed out the factors which contribute to the association rate constant are different from those which contribute to the rate of dissociation. The formation of a drug-receptor complex involves the co-operation of intermolecular forces and these forces result in there being a force field normal to the receptor surface acting upon drug molecules diffusing in the neighbourhood. This field will therefore modify the rate of bombardment of the receptor and will increase the rate if the net force is attractive and decrease it if the net force is repulsive. The rate of association therefore depends on this force field as well as such effects as the probability that the drug is presented in an optimal aspect during its approach to the receptor and the effects of hydration, or ion occupancy. In contrast when the drug dissociates from the receptor it has to escape from the force field but in this case short range forces such as van der Waals are likely to be more important. Therefore if it were possible to measure the rate constants for the antagonist-receptor interaction changes in affinity could be related to changes in these various factors and so a whole new dimension would be added to structure-activity comparisons.

If the rate of onset and recovery from antagonism is limited
by the rate at which the antagonist interacts with the receptors, rather than its access to the receptors, the association and dissociation rate constants could be determined from the observed rates of onset and offset of antagonism. Indeed several workers such as Paton (1961) and Rang (1966) have done this, justifying their assumption on the good agreement found between the observed kinetics of antagonism and what would be expected if interaction was rate limiting.

2) Considering first the onset and recovery from antagonism:

If the kinetics of antagonists are determined by the rate at which they interact with the receptors and if it is assumed that antagonist molecules combine with the receptors thus -

\[
\text{ANTAGONIST} + \text{RECEPTORS} \xrightarrow{k_1} \text{ANTAGONIST-RECEPTOR COMPLEX} \xrightarrow{k_2}
\]

equations can be derived describing the onset and decline of antagonist occupancy:

During the onset of antagonist, \( p \), the proportion of receptors occupied by the antagonist changes with time, \( t \), in the following way,

\[
\frac{dp}{dt} = k_1(1-p)c - k_2p,
\]

\( c \) being the concentration of drug, assumed to be that applied in the bathing fluid. (See also p.44)

Integration of this equation shows that, if a drug concentration, \( c \), is applied at zero time, (when \( t=0 \), \( p_0=0 \)), the proportion of the receptors occupied by the drug rises exponentially to its equilibrium value \( p_\infty = cK_a^\text{eff}/(1 + cK_a^\text{aff}) \), according to the following equation:

\[
P_t = P_\infty \left[1 - \exp\left(-(k_1c + k_2) t \right)\right],
\]
\[ p_t \text{ being the antagonist occupancy at time } t. \]

Similarly when the concentration of drug in the bathing solution is reduced from \( c \) to zero, (when \( t = 0, p_0 = \frac{cK_{aff}}{1 + cK_{aff}} \)), integration of equation 3 shows that \( p_t \) falls exponentially to zero according to the following equation:

\[
P_t = p_0 \exp(-k_2t)
\]

Therefore when interaction is rate limiting:

1. occupancy changes exponentially during both onset and offset,
2. the rate constant for offset, \( -k_2 \), is independent of \( c \), the antagonist concentration, and
3. the ratio of the onset rate constant \( -(k_1c + k_2) \) to the offset rate constant \( -k_2 \) should equal the equilibrium dose ratio since

\[
\frac{k_1c + k_2}{k_2} = \frac{K_{aff}c + 1}{DR_{\infty}}
\]

3) A note on the procedures used to follow antagonism

Therefore in order to compare the observed kinetics of antagonism with the predictions of this model it is necessary to determine how \( p \) changes during the onset and offset of antagonism. The occupancy at any time \( t \) can be calculated from the dose ratio at that time, \( DR_t \), if a suitable technique is used as:

\[
p_t = \frac{DR_t - 1}{DR_t}
\]

Ideally the concentration of agonist would be adjusted throughout so that the responses produced exactly matched that of a known concentration before the antagonist was added. The dose ratio at time \( t \) would then simply be the ratio of the dose at time \( t \) to that used before the antagonist was added. However this ideal situation can never be achieved and so the response sizes vary to a
greater or less extent and this brings two problems.

One is that the size of response produced by a given concentration of agonist is influenced by the size of the preceding response; the depression of responses following a large response, i.e. desensitization, has frequently been observed, (see also P.138). The other problem is that the 'exact' dose ratio has to be calculated from the dose-response relationship. Thus the accuracy with which the dose ratio is estimated is dependent on the degree to which the response sizes are kept constant and the care taken to establish the dose response relationship.

When Rocha e Silva & Beraldo (1948) followed the kinetics of antagonists on guinea-pig ileum, not only did they use a constant dose of agonist throughout together with concentrations of antagonist causing a complete suppression, but also they did not examine the relationship between dose and response. For the reasons given above a reasonable estimate of dose ratio can not be made under these conditions. Rocha e Silva & Beraldo in fact calculated antagonist occupancy, not from the observed dose ratio, but from the percentage by which the control responses were suppressed. Due to such factors as spare receptors, Stephenson (1956), antagonist occupancy can not be determined in this way. It is therefore not surprising that their results are at variance with those of subsequent studies in which more suitable techniques are used.

Rocha e Silva & Beraldo (1948) found that during the onset and recovery from antagonism occupancy did not change exponentially with time and therefore was not in accordance with the predictions of the interaction-limited model. However Paton (1961), Paton & Rang (1965), Rang (1966), studied the kinetics of antagonists on guinea-pig ileum and found that they agreed with the predictions of the interaction-
limited model. As they used a suitable technique in which the agonist dose was adjusted to keep the responses as near the same size as possible, and antagonist occupancy was calculated from the dose ratio determined from the experimentally determined relationship between dose and response, the apparent agreement they found with the predictions of the interaction-limited model can not be attributed to their technique.

4) Paton (1961) studied the kinetics of antagonism of hyoscine, mepyramine and atropine on intact lengths of guinea-pig ileum using acetylcholine and histamine as agonists. The changes in occupancy during onset and offset were found to be approximately exponential, the rate constant of offset was found to be independent of the antagonist concentration, (see also Paton, 1961), and the ratio of the onset rate constant to the offset rate constant was approximately equal to the equilibrium dose ratio.

Paton & Rang (1965) also found agreement between the kinetics of acetylcholine antagonists atropine, methylatropine and lachesine with the predictions of the interaction-limited model. They used longitudinal muscle strips from guinea-pig ileum as did Paton & Rothschild (1965) who found a similar agreement with the antagonists hyoscine and mepyramine.

5) In addition Rang (1966) also found that the kinetics of the interaction between a fast-acting and a slow-acting antagonist agreed with the predictions of the interaction limited model:

If the rate of interaction between a drug and its receptors is rate-limiting and if a fast antagonist is so fast that it is at all times in equilibrium with the receptors not occupied by the
slow antagonist, the proportion of receptors occupied by the slow antagonist, $p_{SL}$, will change with time as follows:

$$\frac{dp_{SL}}{dt} = k_1 SL (1 - p_{SL} - p_F) - k_2 p_{SL}$$

(7)

$SL$ being the concentration of the slow antagonist, $k_1$ and $k_2$ being the slow antagonist-receptor association and dissociation rate constants and, $p_F$ being the proportion of the receptors occupied by the fast antagonist.

If a tissue is equilibrated with a slow antagonist and then a fast antagonist is added, (when $t=0$, $p_{SL} = SL K_{SL} / (1 + SL K_{SL})$), integration of equation 7 shows that $p_{SL}$ will decline exponentially to its equilibrium value $p_{SL,\infty} = SL K_{SL} / (1 + SL K + F K_F)$, thus

$$p_{SL,t} = p_{SL,\infty} - (p_{SL,\infty} - p_{SL,0}) \left[ \exp \left( -k_2 \frac{1 + SL K + F K_F}{1 + F K_F} t \right) \right]$$

(8)

Similarly when the fast antagonist is removed, (when $t=0$, $p_{SL} = SL K_{SL} / (1 + SL K + F K_F)$, $p_F=0$), integration of equation 7 shows that $p_{SL}$ will increase to its new equilibrium value $p_{SL,\infty} = SL K_{SL} / (1 + SL K_{SL})$, thus:

$$p_{SL,t} = p_{SL,\infty} (1 - \exp \left[ -k_2 (SL K_{SL} + 1) t \right])$$

(9)

Rang (1966) investigated the kinetics of interaction between the fast antagonist undecyltrimethyl ammonium and the slow antagonist atropine. As the difference in the rate constants of these two antagonists was not considered to be sufficiently large to assume that the fast antagonist was at all times in equilibrium with the free receptors, an analogue computer was used to predict the occupancy changes. Close agreement was found.
between the predicted changes and those observed experimentally.

Stephenson & Ginsborg (1969) also studied the interaction between a slow and a fast antagonist on guinea pig ileum using the slow antagonist benzylisopropylamine methiodide and the fast antagonist pentyltriethyl ammonium. They followed the interaction using both the agonist pentyltrimethyl ammonium (pentyl TMA), and the less efficacious agonist hexyltrimethyl ammonium (hexyl TMA). They found that although the pentyl TMA was depressed according to the combined occupancy of the slow and the fast antagonists, the responses produced by hexyl TMA could be paradoxically potentiated by increasing the concentration of the fast antagonist, although this increased the combined occupancy. Subsequently, (Ginsborg & Stephenson, 1974), they studied the paradoxical potentiation quantitatively and found that it was consistent with interaction being rate-limiting.

In addition to the kinetics of antagonism on guinea-pig ileum, the kinetics of tetrodotoxin's action on the nonmyelinated fibres of desheathed rabbit vagus nerves, are also apparently compatible with an interaction-limited system; Colquhoun & Ritchie (1972), found that tetrodotoxin's occupancy appeared to change exponentially during the onset and offset of its action, that the rate constant for offset appeared to be independent of the concentration of tetrodotoxin and the ratio of the onset and offset rate constants agreed reasonably well with the equilibrium dose ratio.

However on both these tissues certain observations have been made which are difficult to explain if interaction is rate-limiting. Considering first the kinetics of muscarinic antagonists on guinea-pig ileum; there are four observations which indicate that access may
in fact be rate-limiting at least in certain circumstances.

1. Paton (1961) observed that atropine washed out from intact pieces of ileum with a half time of 40 minutes whereas Paton & Rang (1965) found that atropine washed out from longitudinal muscle strips prepared from guinea-pig ileum with a half time of about 7 minutes, the equilibrium dissociation constant not being significantly different. As it seems very unlikely that the chemical nature of the receptor would differ between the two preparations, especially as the equilibrium constants were not significantly different, the slower offset of action of atropine when intact pieces of ileum were used must have been due to some sort of access limitation.

2. Paton (1961) also observed that although the kinetics of action of hyoscine below a 100 fold antagonism could be satisfactorily represented as an exponential process determined by two constants only, recovery after higher concentrations was very considerably delayed. Therefore the rate of offset following these higher concentrations must also be access limited.

3. The results of Thron & Waud's (1968) study of the kinetics of atropine on longitudinal muscle strips of guinea-pig ileum are also difficult to explain in terms of the interaction-limited model. They investigated the re-establishment of atropine's occupancy after washing out the 'fast' antagonist N-methyl-N-(3-diphenylaminopropyl) piperidinium bromide (MDP), the strips having been previously equilibrated with a mixture of atropine and MDP. They found that the results varied considerably from one preparation to another, in some cases the reoccupation of the receptors by atropine appeared to be slow and in others fast. Such variability is difficult to explain in terms of the interaction-limited model as the behaviour
would depend on the chemical rate constant which should differ little from one preparation to another. In contrast, the behaviour of access-limited systems would be expected to vary from one preparation to another according to the geometry of the tissue.

4. Thron & Maud (1968) also observed that previous treatment of longitudinal muscle strips with dibenamine, accelerated both the onset and the offset of atropine's action, although the equilibrium dissociation constant was not significantly altered. This observation is also difficult to explain in terms of the interaction-limited model.

8) In addition there is one other observation which has been claimed to indicate that interaction can not be rate limiting. This observation is that although recovery from many antagonists is slow, they appear to equilibrate with agonist molecules in a very short time. For instance, Gaddum (1937) noted that if a sufficient concentration of adrenaline is applied to a piece of rabbit uterus which has been immersed in ergotamine, a maximal contraction occurs in less than one minute. On the other hand, if a piece of uterus is immersed in a solution of ergotamine at least one hour is required before the action of this drug is complete and washing out is also slow. He therefore concluded that this cannot be due to a slow dissociation from the receptors because of the apparently fast dissociation in the presence of adrenaline, and must therefore be due to some other factor such as slow diffusion through the tissue to the receptors.

This conclusion is based on two faulty assumptions. In the first place he assumed that the agonist occupied all the receptors when a maximal contraction was produced. It is now realized that a potent agonist can produce a maximal contraction while occupying only a small proportion of the receptors, (Stephenson, 1956). Therefore
the ability of adrenaline to produce a maximal contraction in less than one minute does not mean that the ergotamine has dissociated from the receptors during this short time. As Rang (1966) pointed out, if the agonist occupancy is negligible in relation to the available receptor pool the antagonism may appear to be competitive even if no dissociation of the antagonist takes place during the short exposure to the agonist.

Gaddum also assumed that the rate of dissociation of the antagonist in the presence of an agonist is equal to the rate of dissociation when the antagonist is washed out. Rang (1966) pointed out that if the rate of dissociation of the antagonist is rate-limiting, the antagonist occupancy will decline exponentially when the antagonist is removed from the bathing solution with a rate constant of $-k_2$, whereas in the presence of a concentration $A$ of agonist, affinity $K_A$, the antagonist's occupancy will decline exponentially with a rate constant of $-k_2 (SL K_{SL} + A K_A + 1) / (A K_A + 1)$, from equation 8. Therefore if the agonist concentration is adjusted so that the agonist occupancy at equilibrium, $p_A^*$, is kept constant, (corresponding to the standard response at which the dose ratio is measured), this rate constant then equals $-k_2 (SL K_{SL} + 1) / (p_A^* SL K_{SL} + 1)$. This means that if the agonist occupancy necessary to produce a standard response is small then the rate of adjustment of antagonist occupancy may be faster than if the rate constant were $-k_2$. Thus a fast rate of dissociation in the presence of adrenaline is not necessarily incompatible with a slower rate of offset when the antagonist is washed out.

Furchgott (1955) made a similar false assumption when he considered the kinetics of antagonists on rabbit aortic strips. He observed the progressive blockade of sympathomimetic drugs produced by raising the concentration of the potent reversible competitive
antagonists dihydroergotamine and phentolamine and compared this progressive blockade with that produced by the 'irreversible' antagonist dibenamine. Progressive blockade with dibenamine initially produced a parallel shift of the log dose response curve. Then if the level of blockade was increased above a certain level, the agonist was no longer able to produce a maximum contraction and the slope of the log dose response curve was decreased. In contrast he found that if progressive blockade produced with a potent reversible antagonist was followed, the parallel shift of the log dose response curve greatly exceeded that found on progressive blockade with dibenamine.

Furchgott argued that if the rate of recovery was limited by the rate of dissociation of the antagonist-receptor complex, a potent reversible antagonist would act essentially like an irreversible antagonist. Therefore the parallel shift of the log dose response curve produced by progressively raising the concentration of the irreversible antagonist should equal that found if a potent reversible antagonist was used. As this was not found to be the case, Furchgott concluded that recovery from antagonism can not be limited by the rate of dissociation of the antagonist-receptor complex. However, as pointed out above, potent reversible antagonists would not necessarily be expected to be essentially irreversible in the short exposure time to a potent agonist. This therefore could explain Furchgott's discrepancy.

Despite this confusion the other observations do suggest that access may be the rate-limiting step determining the kinetics of antagonists on guinea-pig ileum. Similarly the kinetics of tetrodotoxin's action may also be access limited despite the apparent
agreement with the interaction-limited model. Although Colquhoun & Ritchie (1972) found that the kinetics of tetrodotoxin's action on this tissue was compatible with the interaction-limited model, they found that recovery took place with a time constant of about 40 minutes. This contrasts with Hille's (1970) finding that the recovery from tetrodotoxin's action on frog-nodes was complete within 15 seconds, which suggests that the toxin-receptor interaction is much faster than the rates observed by Colquhoun & Ritchie.

In addition other isolated tissues have been investigated and the kinetics of antagonists on these tissues do not appear to be interaction-limited.

The kinetics of adrenergic antagonists on rabbit aortic strips

Furchgott (1955) followed the recovery of the sensitivity of rabbit aortic strips to adrenaline and noradrenaline following exposure to various concentrations of dihydroergotamine for various lengths of time. He used a dose ratio method and found that, contrary to the predictions of the interaction-limited model, the dose ratio changed exponentially with time. Also blockade was found to develop at a rate similar to that of recovery and the rate at which the antagonist diphenhydramine blocked histamine was similar to the rate at which it blocked noradrenaline.

The kinetics of adrenergic antagonists on rabbit fundus

Paton (1967a) followed the kinetics of the antagonism produced by piperoxane, yohimbine, dihydroergotamine, phentolamine and tolazoline to noradrenaline using strips of rabbit fundus. Although the offset of occupancy was usually exponential as predicted by the interaction-limited model, the rate of offset became slower if the
antagonist concentration, or the time the tissue was exposed to the antagonist, was increased. In addition the kinetics of offset of phentolamine's occupancy was not convincingly exponential.

He also followed the kinetics of the interaction between piperoxan, a fast antagonist, and dihydroergotamine, a slow one. He found no hint at all of the transient 'overshoot' or 'undershoot' of total antagonist occupancy which would be expected to occur if interaction was rate-limiting.

The kinetics of competitive antagonists on the frog neuromuscular junction

Waud (1967) followed the rate of action of the competitive neuromuscular blocking agents tubocurarine, dimethyltubocurarine, gallamine and $n$-$C_{10}$-$21$-$NMe_3$ applied iontophoretically, (or by changing the concentration in the bathing solution), to the end-plate region of frog skeletal muscle fibres. The rate of action of these drugs was measured by testing the end plate with iontophoretically applied doses of carbachol.

When tubocurarine was added by infusion at a known rate into the Ringer's solution flowing at a constant measured rate through the muscle chamber and a constant dose of carbachol was used to follow the antagonism, the rate of offset from 2.5 $\mu$M was found to be slower than from 1 $\mu$M, and the rate of onset of 1 $\mu$M was not much faster than the rate of offset. Similar results were also found when the antagonists were applied iontophoretically, or when the dose of carbachol was adjusted to maintain the response size.

In addition the four antagonists examined were found to all act at about the same rate although the mono-quaternary ion is considerably weaker than the others and relatively non-specific.

None of these observations are consistent with interaction being rate limiting.
The kinetics of muscarinic antagonists on guinea-pig heart

Thron & Waud (1968) studied the rate of action of atropine in isolated guinea-pig atria and perfused hearts (Langendorff) using the agonist carbachol. Although the onset and offset of atropine in isolated atria and perfused hearts was qualitatively as predicted by the interaction-limited model, when perfused hearts were used the rates of both onset and offset were faster than when isolated atria were used and varied considerably from one perfused heart to another. In one of the faster preparations the effect of atropine was almost as fast as that of carbachol.

In addition they followed the kinetics of the interaction between butyrylcholine and atropine. When butyrylcholine, a fast antagonist, was added after equilibration of the tissue with atropine, there was only a small initial overshoot lasting no more than a minute or two.

In this tissue too therefore the kinetics of antagonists do not appear to be interaction-limited.

The kinetics of antagonists of histamine on guinea-pig ileum

In addition to following the kinetics of cholinergic antagonists, Paton (1961) also followed the kinetics of mepyramine's antagonism of histamine. He found that although below a 20-fold antagonism the kinetics of antagonism could be satisfactorily represented by an exponential process determined by two rate constants only, recovery after higher concentrations was considerably slower. Recovery following higher concentrations must therefore be access limited.

These studies show that the kinetics of antagonists on many tissues do not appear to be interaction-limited. This being the
case it would seem unlikely that the kinetics of antagonists on
guinea-pig ileum would be, especially as some of the other studies
involved the application of the antagonist iontophoretically.

However in spite of the evidence that the kinetics of
antagonists on many tissues are access limited, several workers
have come to the opposite conclusion because the access limited
models, they considered to be the most appropriate, did not
appear to account for their observations. For instance,
Del Castillo & Katz (1957) concluded that the rate of action of
curare at motor end plates must be interaction limited as diffusion
could not account for its slowness compared with acetylcholine or
carbachol. Similarly Rang (1966) discounted access because his
limited biophase model would not predict an exponential relationship
between occupancy and time during the onset and offset of antagonism
AND ALSO the overshoot and undershoot of total occupancy when a
fast antagonist is superimposed onto a slow antagonist.

Unfortunately, in principal, it is not possible to prove that
interaction is rate-limiting from discrepancies between an access
limited model and experimental observations. This is because of
the large number of unknown factors which could influence the kinetics
of antagonism. Thus discrepancies between experimental observations
and an access limited model could just mean that the 'correct'
model was not being considered.

On the other hand if an access model could provide an
explanation for the apparent agreement with the interaction-limited
model, and the observations which are not consistent with the
interaction-limited model, this would substantially support the
hypothesis that the kinetics of antagonists on guinea-pig ileum are
access limited, the main objection to access being rate-limiting on this tissue being the apparent agreement of the kinetics of antagonists and the interaction-limited model and the lack of an access model which was consistent with the experimental observations. Various access-limited models will therefore now be considered.

12) Simply diffusion

The simplest possibility is that the rates reflect different rates of diffusion. If a drug is administered to the bathing fluid surrounding an isolated tissue in an organ bath, the drug molecules move from the bathing fluid to the receptors by free-diffusion. Similarly when the drug is washed away, the drug molecules diffuse away from the receptors into the bathing fluid. Therefore if the rate of interaction of the drug with the receptors is sufficiently fast, diffusion between the bathing fluid and the receptors will be the rate-limiting step.

The mathematical theory of diffusion is based on what is known as Fick's first law of diffusion. This law was first formulated by direct analogy with the equations of heat conduction and it states that the rate of transfer of a diffusing substance through unit area of a section in an isotropic medium is proportional to the concentration gradient measured normal to the section:

\[ Q = -D \frac{\partial c}{\partial x} \]

Fick's first law

where \( Q \) is the rate of transfer per unit area of section, \( c \) is the concentration of diffusing substance,
x is the space co-ordinate measured normal to the section, D is the diffusion coefficient, and an isotropic medium is one whose structure and diffusion properties in the neighbourhood of any point are the same in all directions.

This equation is not restricted to any particular pattern of diffusion or geometrical arrangement of concentration gradients, for it describes only what is happening in an infinitesimal volume of solution during an infinitesimal interval of time. In order to calculate the actual changes in concentration which occur through measurable distances and during finite intervals of time, this equation must be integrated which is a complex process and involves the assumption of a specific geometrical arrangement. Specific solutions for a number of geometrical arrangements have been worked out, Crank (1956), and conveniently it is found that the solution applying to diffusion in one dimension into a plane sheet approximates to diffusion into a cylinder if the thickness of the cylinder is small compared to its diameter. Therefore the same solution can be used to describe diffusion into rat diaphragm, which approximates to a plane sheet, as to describe diffusion into guinea-pig ileum, a cylindrical tissue.

For diffusion into a plane sheet the time t needed to attain at a point 1 any specified fraction of its final equilibrium concentration is inversely proportional to the diffusion coefficient and directly proportional to the square of the distance through which it must diffuse:

$$t \propto \frac{1^2}{D}$$

The proportionality 'constant' is itself a function of the particular fraction of equilibrium and values can be obtained from a number of sources, see Riggs (1963).
For instance Cuthbert & Dunant (1970) used Olson & Schultz's (1942) tables showing how the temperature in a solid changed during heating or cooling, this approach being similar to the way in which Fick formulated his diffusion laws by direct analogy with the equations of heat conduction. In this way they obtained an analytical solution showing the relationship between \((d/d_{a})\) and \((Dc/1^2)\), \(d\) being the concentration at a point \(1\), at a time \(t\), in analytical units of concentration, i.e. \(c_{1}/k_{2}\), and \(d_{a}\) being the concentration in the bulk of the bathing solution, also in analytical units.

Paton & Waud (1964) obtained a similar analytical solution of Fick's equation but they used a resistance capacitance analog after conversion to a finite differences approximation. This finite differences approximation is very similar in essence to Thron's (1972) linear multicompartment model.

Thron (1972) considered the space between the receptors and the bathing medium to be divided into compartments so small that within any compartment the concentration of diffusing substance is uniform. As the unidirectional rate of outward diffusion of a substance from any compartment is directly proportional to its concentration in that compartment, (Fick's law), such a system can be described by a set of linear differential equations.

He then showed that if the tissue, equilibrated with some applied drug concentration, is abruptly exposed to a new constant drug concentration, then as the concentration \(c_{i}\) in the \(i\)th compartment changes from its initial value \(c_{i,0}\) to its final steady state value \(c_{i,\infty}\), the function \((c_{i} - c_{i,0}) / (c_{i,0} - c_{i,\infty})\) decreases from 1 to 0, following a time course which is independent of the initial and final drug concentrations.

A similar sort of qualitative description to describe the
change in concentration in the proximity of the receptors during the onset and offset of drug action, can also be deduced from the following simple picture:

If a drug is added to the bathing solution at time $t = 0$ so that at this point in time the concentration in the bathing fluid is $c_a$ whereas that in the proximity of the receptors is zero,

at equilibrium, $t \rightarrow \infty$, the concentration in the bathing solution will still be $c_a$ but that in the proximity of the receptors will be $c_a$ also.

Similarly, if the drug is removed from the bathing solution at time $t = 0$, at this point in time the concentration in the bathing solution will be zero while the concentration in the proximity of the receptors will still be $c_a$.

At equilibrium, $t \rightarrow \infty$, the concentration in the bathing solution will still be zero but that in the proximity of the receptors will be zero also.

Thus in both cases the concentration gradient at time $t = 0$ is $(c_a - 0)$ and that at time $t \rightarrow \infty$ is zero. Therefore as Fick's law states that the rate of transfer of diffusing substance is proportional to the concentration gradient, the rate of rise of concentration in the proximity of the receptors during onset will be similar to that during offset. This qualitative description is thus very similar to that obtained more precisely earlier.
For antagonists, the concentration in the proximity of the receptors at any time, \( c_{b,t} \) can be calculated from the dose ratio at that time, \( \text{DR}_t \) as:

\[
\text{DR}_t - 1 = c_{b,t} K_{aff},
\]

i.e. equ. (2) if it is assumed that the receptors are at all times in equilibrium with the concentration \( c_b \). Therefore if a suitable technique is used (as discussed earlier), and if the antagonism is sufficiently slow, the rate at which the concentration changes can be compared with the qualitative predictions of a diffusion limited system.

Alternatively the rate of change of dose ratio can itself be used. As dose ratio is a linear function of the antagonist concentration, the rate of change of dose ratio during onset,

\[
\frac{(\text{DR}_\infty - \text{DR}_t)}{(\text{DR}_\infty - 1)},
\]

should be similar to that during offset,

\[
\frac{(\text{DR}_t - 1)}{(\text{DR}_\infty - 1)},
\]

where \( 1 \) is the dose ratio in the absence of antagonist, \( \text{DR}_t \) is that at time \( t \), and \( \text{DR}_\infty \) is that at equilibrium with the antagonist. This compares with the complex way in which occupancy will change if diffusion is rate-limiting, because occupancy is related to concentration in the following way:

\[
\frac{p_t}{c} = \frac{c_t K_{aff}}{1 + c_t K_{aff}},
\]

i.e. equ. (1)

Of those few studies in which a suitable technique is used, only that of Furchgott (1955) using rabbit aortic strips seems to be compatible with a diffusion limited system. (See section on biophase models, p.42). The kinetics of all the other antagonists, sufficiently slow to be studied in this way, do not agree qualitatively with a diffusion-limited system.

In addition it is possible to make certain quantitative predictions for a diffusion limited system, if certain assumptions are made. Although these predictions are only tentative because of
the assumptions, the kinetics of slow antagonists do not seem to be explicable in terms of diffusion only. This sort of calculation is demonstrated below.

Holmes, Jenden & Taylor (1951) considered the kinetics of tubocurarine added to the bathing solution of isolated rat diaphragm preparations. They assumed that drug molecules penetrate a tissue through the extracellular spaces and that the diffusion coefficient is constant within the extracellular spaces and is equal to that in dilute aqueous solutions.

They calculated the diffusion coefficient from Thovert's equation: the ratio of the diffusion coefficients of two substances is inversely proportional to the ratio of the square roots of the molecular weights. Thus from the diffusion coefficient of sucrose they calculated that of tubocurarine to be about $3.0 \times 10^{-6} \text{ cm}^2/\text{sec}$.

In addition they estimated that although the tissue is about 0.06 cm thick, the actual diffusion path will be double this and that because only the extracellular spaces are available for diffusion only 0.15 of the total area will be available for diffusion. These estimations were made from geometric considerations.

They then used a relationship derived by Hill (1928) for the time, $t$, taken from the concentration of diffusing substance within a sheet of tissue to reach an average of 50% of its outside concentration, the sheet being exposed on both sides to the diffusing substance:

$$t = \frac{0.196 \times l^2}{D},$$
where \( t \) is the time in seconds,
\( D \) is the diffusion coefficient in cm\(^2\)/sec
and \( 2l \) is the thickness of the tissue in cm.

They therefore estimated that for tubocurarine,

\[
t = \frac{0.196 \times (2 \times 0.03)^2}{3 \times 10^{-6} \div 0.15} = 35 \text{ seconds.}
\]

They then determined \( t \) experimentally assuming that at equilibrium the concentration of antagonist in the proximity of the receptors is the same as that in the bathing solution: If at equilibrium a certain concentration of antagonist, \( c \) say, produces a certain degree of block, the time taken for a concentration of \( 2c \) to reach this degree of block will be equal to \( t \). \( t \) was found in this way to be about 40 minutes.

As this is some 60 times slower than that predicted if diffusion was rate limiting, they therefore concluded that the rate of action of curare could not be accounted for in terms simply of diffusion.

By making a similar series of assumptions and using their analogue solution of Fick's law, Paton & Waud (1964) predicted the rate of rise of concentration of atropine inside the longitudinal muscle of guinea pig ileum. As they found a discrepancy, similar to that found by Holmes, Jenden & Taylor (1951), they also concluded that the rate of action of atropine could not be accounted for in terms of simple diffusion.

In conclusion therefore, the kinetics of slow antagonists have not been found to agree qualitatively with the predictions of a diffusion limited system, and in addition estimations of diffusion
rates predict rates of antagonism considerably faster than those found experimentally. Although the assumptions on which these calculations are based could be grossly out, it seems more likely that the kinetics of these antagonists is not limited simply by diffusion.

14) If an antagonist is so fast that its rate of action cannot be followed no qualitative comparisons can be made. In addition the rates of action of fast antagonists such as undecyltrimethyl ammonium, Rang (1966), is a matter of seconds rather than minutes and so could perhaps be accounted for qualitatively in terms of diffusion.

Similarly the rate of action of most agonists is a matter of seconds and could therefore also be accounted for in terms of diffusion. In contrast to fast antagonists though it is possible to examine the rate of action of agonists qualitatively if the tissue response is not the rate limiting step. For instance Cuthbert & Dunant (1970) examined the rates of action of acetylcholine, carbachol and histamine on guinea-pig ileum in the following way, which they called a transient analysis:

They assumed that if a submaximal concentration $a'$ of agonist produced a response $r$, then the time taken for the response to a supramaximal concentration $a''$ to reach $r$ would be the time taken for the concentration in the proximity of the receptors to rise to $a'$, i.e. $a'/a''$ of that in the bathing solution. (This is very similar to Holmes, Jenden & Taylor's (1951) assumptions for antagonist kinetics.)

Knowing that time $t$, and the value of $a'/a''$, $D/l^2$ can be calculated from their analytical solution of Fick's law showing the relationship between $a'/a''$ and $Dt/l^2$. 
Knowing $D/1^2$, the concentration in the proximity of the receptors at various times can be determined and the response produced by these concentrations inferred from the transience of the supramaximal response.

If the rate of action of the agonist is limited by diffusion, the relationship between concentration and response as obtained above should be indistinguishable from that obtained in the conventional manner, i.e., from responses to known concentrations of agonist.

This was found to be the case for the actions of acetylcholine, carbachol, and histamine on guinea-pig ileum at 35-37°C, and also for the depolarizing action of acetylcholine on the isolated rat sympathetic ganglion preparation. They also found that when the rates of action of the different agonists were compared, the faster ones were those which would be expected to diffuse more rapidly from molecular weight considerations.

This type of analysis did however fail for the action of acetylcholine on frog rectus abdominis muscle and on the dorsal muscle of leech. As the responses produced by both these preparations are slow, it is possible that for these preparations, the production of a response is the rate-limiting step rather than the rise in concentration in the proximity of the receptors. Therefore the use of a response, such as depolarization, nearer to the drug-receptor interaction might give a different result.

In addition to these studies Del Castillo & Katz (1955) also found that the rate of action of acetylcholine was consistent with its being diffusion limited. They predicted the time course and size
of depolarization of acetylcholine applied iontophoretically to frog sartorius end plates assuming that diffusion was the rate-limiting step and this was found to agree with that observed.

15) From these comparisons it therefore seems that although the rate of action of agonists, in the absence of any antagonist, appears to be diffusion-limited and the rate of action of fast antagonists may be diffusion-limited, the rate of action of the slow antagonists does not appear to be. Any explanation of the rate of action of the slow antagonists must therefore be compatible with these differences.

There is a general relationship between the potency of competitive antagonists, as measured by the concentration required to produce a given degree of antagonism, and the rate at which the antagonism wears off when the antagonist is removed from the bathing fluid. The more potent compounds are also the slower.

There is a similar relationship between speed and concentration when agonists and antagonists are compared. For instance Paton & Rang (1966) observed that although methylfurmethide had a molecular weight close to that of lachesine it was some hundreds of times faster. This difference can not be due to one being an agonist and the other an antagonist because the fastest antagonists act at rates comparable with those of agonists. In addition when the kinetics of different antagonists are compared the relationship between concentration, receptor occupancy and speed is similar to that found when agonists are compared with antagonists. This implies that agonists act quickly, not because they are agonists, but because they occupy only a very small proportion of the receptors to produce their effect and because of their affinity for the receptors, relatively large concentrations are required to produce this occupancy.

The problem is therefore, not why do agonists act faster than
antagonists, but why is there the apparent relationship between the speed of a drug and its 'potency'. (The relationship between affinity and efficacy is a different problem altogether.)

If the rate of action of antagonists is diffusion-limited the difference in rates would not be expected on molecular weight considerations.

There is however another access limited model which has been considered in some detail and this involves the concept of a biophase.

**The biophase model**

16) The concept of a biophase was first used in connection with the activity of narcotics; Ferguson (1939) considered the distribution of narcotics to be between two heterogeneous phases, the external circumambient phase (solution or vapour) in which the narcotic was applied, and the phase or surface layer which is the seat of toxic action. This latter phase he called the biophase. He suggested that physically toxic substances should be compared, not by their concentration in the external solution or vapour, but by their relative concentrations in the biophase, as estimated by their chemical potential in the external solution or vapour. In addition, as the potency of substances acting by a physical mechanism would thus be related to their partition between the external phase and the biophase, substances with the greatest potency, i.e. highest partition coefficients, would be washed out the slowest.

On these criteria Fastier & Reid (1952) noticed that the action of alkyl-isothioureas were apparently consistent with their having a physical mechanism. They observed the relationship between the
potency and the length of the alkyl side chain, and the relationship between potency and the time for recovery of sensitivity to agonists on various preparations, such as perfused rat hind quarters, guinea-pig ileal strips, amine oxidase liver suspensions. They found that activity increased in a geometrical progression with chain length, this being very similar to the way in which Meyer & Hemmi (1935) had found the narcotic activity of n-aliphatic alcohols varied with chain length. They also observed that recovery from the more potent compounds was slower than recovery after the less potent compounds. They therefore suggested that the differences in potency were perhaps not due to differences in effectivity at the site of action, but due to their relative partition coefficients between the aqueous phase in which they were applied and the biophase.

Furchgott (1955) mathematically developed this idea that the rate of action of drugs is related to their partition between the aqueous phase, in which they are applied, and a biophase. He visualized the following sort of scheme:

\[
\begin{align*}
\text{AQUEOUS PHASE} & \quad \text{BIOPHASE} \\
c_a & \quad \overset{k_{\text{in}}}{\longleftrightarrow} \quad k_{\text{out}} \quad c_b
\end{align*}
\]

He assumed that the concentration of drug in the biophase is uniform and equal to \( c_b \), and also that the concentration of drug in the aqueous phase is uniform and equal to \( c_a \).

Also he assumed that the rate of entry into the biophase is proportional to \( c_a \) and the rate of escape, proportional to \( c_b \), the rate constants governing entry and escape being \( k_{\text{in}} \) and \( k_{\text{out}} \).

Therefore, the rate of entry \( = k_{\text{in}} c_a \), and the rate of escape \( = k_{\text{out}} c_b \).
Having defined $k_\text{in}$ and $k_\text{out}$ so that they refer to the rate of transfer through an area corresponding to a biophase volume of one unit, and assuming that the quantity of drug taken up by the receptors is negligible compared to the volume of the biophase, therefore the biophase concentration changes with time $t$, in the following way:

$$\frac{dc_b}{dt} = k_\text{in} c_a - k_\text{out} c_b$$

Thus when the concentration of drug in the aqueous phase is raised from zero to $c_a$, (when $t = 0$, $c_b = 0$), integration of this equation shows that the biophase concentration approaches its equilibrium value $c_{b,\infty} = (k_\text{in}/k_\text{out}) c_a$, according to the following equation:

$$c_{b,t} = c_{b,\infty} \left(1 - \exp\left[-k_\text{out} t\right]\right)$$

Similarly when the concentration of drug in the aqueous phase is reduced from $c_a$ to zero, (when $t = 0$, $c_{b,t} = c_{b,\infty}$), integration of equation 12 shows that the biophase concentration falls from $c_{b,\infty}$ to zero according to the following equation:

$$c_{b,t} = c_{b,\infty} \exp\left(-k_\text{out} t\right)$$

For drugs acting on receptors, the receptors are considered to equilibrate with the concentration of drug in the biophase.

Therefore if the rate of interaction with the receptors is sufficiently fast, the overall rate of action will be limited by the rate of change of the biophase concentration. In this case, for a competitive antagonist, as the dose ratio is a linear function of the biophase concentration, $\text{DR}_c - 1 = c_{b,t} \text{ Ka}_\text{eff}$, therefore, for the onset of antagonism, $(\text{DR}_{\infty} - \text{DR}_c) = (\text{DR}_{\infty} - 1) \exp(-k_\text{out} t)$.
and similarly for offset, \( (\text{DR}_e - 1) = (\text{DR}_\infty - 1) \exp(-k_{\text{out}}t) \) (16)

Occupancy on the other hand would be related to the biophase concentration-\( [\text{p}_c = \frac{c_{b,t}^t K_{\text{aff}}}{1 + c_{b,t}^t K_{\text{aff}}} ] \), and so occupancy would change in a complex fashion when \( c_{b,t} \) changed exponentially.

Therefore dose ratio would change during the onset and offset of antagonism exponentially and with the same rate constant and would be indistinguishable qualitatively from a system limited by simple diffusion. The biophase model could thus be considered as a mathematical approximation to a diffusion limited system. In this case, \( k_{\text{in}} = k_{\text{out}} \) and so

\[
\frac{dc_b}{dt} = k (c_a - c_b)
\]

This is very similar to Fick's equation,

\[
Q = -D \frac{\delta c}{\delta x}
\]

However Fick's equation applies to the rate of transfer per unit area of section across which diffusion takes place, and the biophase model to the change in concentration in a unit volume of biophase, i.e., \( k \) and \( D \) are in different units.

Therefore, for the reasons given when considering diffusion, the kinetics of potent antagonists can not be accounted for by the biophase model.

17) However the biophase model, in addition to giving a mathematical approximation of a diffusion limited system, would also describe an access limited system in which the receptors were separated from the bathing solution by a barrier to diffusion. There is the problem though of finding a physical counterpart to the postulated barrier. Although in a few tissues there is a possible candidate, such as the
connective tissue sheath around a rat's superior cervical ganglion, in many tissues there is no obvious barrier. In these cases the only other possibility would seem to be the cell membrane itself.

At a first glance the cell membrane appears to be unlikely in cholinergic systems at least, because of the evidence that the receptors are exposed to the outside of the cell membrane. The evidence for this is that many quaternary compounds are active at these sites and also because Del Castillo & Katz (1955) found that when acetylcholine was introduced iontophoretically to the inside of frog sartorius muscle cells it had no action.

A closer look at the membrane though suggests that it might incorporate its own barrier. Robertson (1958) stressed that although the basic structural unit of the cell membrane was the typical trilaminar structure seen on the electron micrographs, additional structures were also closely associated with it. For instance, electron micrographs of smooth muscle cells, e.g. Caesar, Edwards & Ruska (1957), and also other cells, show that outside the cell membrane is a relatively thin electron dense layer which is called the basement membrane or basement lamina and this appears to be separated from the cell membrane by a 'light' layer. This lamina appears to be homogeneous or faintly fibrillar in nature and chemical studies show that it appears to be partly composed of acid mucopolysaccharides, Gasic & Berwick (1963).

This basement membrane could therefore act as a barrier to the diffusion of antagonist molecules between the extracellular space and the receptors. However the existence of a barrier would not explain the relationship between the potency of a drug and the rate at which it acts, (P.36), unless it was postulated that it could discriminate between drugs according to their affinity for the receptors. Neither would it account for the apparently exponential
relationship between occupancy and time during the onset and offset of antagonists on guinea-pig ileum.

18) The involvement of a barrier does seem more likely though when considering adrenergic antagonists. As noted earlier, Furchgott (1955), dose ratio appears to change exponentially during recovery from dihydroergotamine and during onset dose ratio appears to develop exponentially at a similar rate. This therefore agrees qualitatively with the predictions of both the biophase model and the diffusion-limited model. The possibility that the biophase-barrier model could be more appropriate comes from Bevan's (1960) investigations into the rate of action of (-epinephrine on rabbit aortic strips.

Bevan (1960) did some calculations, very similar to those of Holmes, Jenden & Taylor (1951), for the rate of action of (-epinephrine on rabbit aortic strips.

He calculated the time t for aortic strips to become 50% saturated with (-epinephrine using the same assumptions concerning diffusion coefficient, the geometry of the tissue and the area available for diffusion as Holmes, Jenden & Taylor (1951) had.

\[ t = \frac{0.196 \times 1^2}{D} = \frac{0.196 \times (2 \times 0.04)^2}{(8.1 \times 10^{-6} \times 0.15)} = 23 \text{ seconds} \]

He then determined t experimentally as had Holmes, Jenden & Taylor (1951). If a certain concentration of agonist produced a certain sized contraction at equilibrium, t is the time taken for the contraction produced by double that concentration to reach that size. t was found to be about 120 minutes, i.e. considerably slower than that expected if diffusion were rate limiting.
In addition he also studied the variation in the rate of contraction at different temperatures between 16°C and 39°C. At a particular temperature, knowing the response r produced by a concentration c₁ of agonist, he then added cᵢ and recorded the slope of contraction at a response height r. He then repeated this process for various concentrations and temperatures.

He then said that if diffusion is the rate-limiting process determining the rate of contraction, and as diffusion is proportional to the concentration gradient, the slope S should be proportional to the concentration gradient (cᵢ - c₁), and S/(cᵢ - c₁) is a measure of the velocity of the process.

As the velocity of a process can be related to the energy of activation of the process by the following equation:

\[ V = A e^{-\frac{E_A}{RT}} \]

Arrhenius equation \quad (17)

where V is the velocity of the process,

\( E_A \) is the activation energy of the process,

T is the absolute temperature, and

R is the gas constant.

As predicted by this equation, when log \( S/(cᵢ - c₁) \) was plotted against 1/T, a linear relationship was found. \( E_A \) was calculated from the slope of this line and was found to be in the order of 37,000 cal/mol/°C.

Knowing that the activation energy of diffusion of many molecules in aqueous solution is the same or very close to the activation energy of viscous flow in water, (about 5,000 cal/mol/°C), whereas the activation energy of diffusion through membranes is
considerably higher than that in aqueous solutions, Danielli & Davson (1935), Bevan therefore suggested that the rate of action of 1-epinephrine might involve diffusion through the cell membrane, thus accounting for the high activation energy and the discrepancy between the calculated and observed values of the Diffusion Coefficient.

Paton (1967) also considered the involvement of a diffusion barrier in connection with the kinetics of adrenergic drugs on rabbit fundus. As described earlier (p.23) his observations did not agree with the interaction-limited model and in particular he found that the kinetics of offset of antagonism varied with the duration of exposure. In addition adrenergic compounds are known to be rapidly and substantially taken up by tissues, the responses to adrenaline and noradrenaline are slower than responses to acetylcholine and there are not many quaternary compounds active at adrenergic receptors. All these observations suggested that adrenergic receptors might not be exposed on the outside of the cell membrane. However he also noted that the observation of Schild (1963), that calcium lack had a parallel effect on acetylcholine and adrenaline responses on rabbit uterus, would be difficult to explain if the adrenergic receptors were on the inside of the cell membrane and the cholinergic receptors on the outside.

19) The concentration of a drug to which the receptors are exposed

In addition to the possible involvement of a diffusion barrier, there is another implication of the biophase model which distinguishes it from simple diffusion. As noted by Furchgott (1955) the biophase concept implies that the concentration of drug with which the receptors are in equilibrium might not be the same as that in the bulk of the bathing solution, i.e. if $k_{in} \neq k_{out}$. Indeed
Fastier & Reid (1952) suggested that the relative potencies of the isothiourea compounds they were investigating might be determined by the degree to which they accumulated near the receptors rather than their affinities for the receptors. This extreme possibility seems unlikely to apply to antagonists on guinea-pig ileum because the observed relationship between dose ratio and time is not consistent with such a model and also factors which influence the kinetics of antagonism (such as calcium ion concentration) do not always cause corresponding changes in the equilibrium dose ratio, Paton & Rothschild (1965).

In addition extreme stereospecificity is exhibited by atropine-like antagonists of acetylcholine, e.g. Long, Luduena, Tullar & Lands (1956).

This therefore leaves the possibility that the potency of an antagonist is partly determined by the degree to which it accumulates near the receptors, and partly by its affinity for the receptors. One would have thought that it would be possible to investigate this by comparing the volume of distribution of quaternary compounds, calculated assuming that their concentration was uniform throughout the extracellular space and that they were not able to penetrate the cell membrane, with the extracellular volume calculated by other means. If these separate estimations agreed it would be reasonable to infer that the concentration of drug to which the receptors were exposed was equal to that in the bathing solution.

Such a comparison can be made from the studies of Krenjević & Mitchell (1960), in which they equilibrated isolated rat diaphragms in solutions of $(5 \times 10^{-4} M)$ acetylcholine for 1-2 hours. They found that 80-90% of the acetylcholine escaped at a rate expected for diffusion through dilute aqueous solutions and that the space corresponding to this free-acetylcholine agreed with the previous estimates of the inulin space. This would therefore seem to indicate
that the concentration of acetylcholine at equilibrium in the extracellular space was the same as that in the bathing solution.

However the issue is complicated by the 10-20% slowly diffusing fraction. If it is accepted that the acetylcholine is not able to penetrate the cell membrane, this fraction is probably also extracellular. This is supported by the fact that inulin appears to underestimate the true extracellular space, Goodford & Leach (1966), perhaps because it is excluded from the space occupied by substances like hyaluronic acid, Ogston & Phelps (1961). In addition as the basement membrane appears to be of a mucoprotein nature, Gasic & Berwick (1963) it may correspond to the space from which inulin is excluded and by inference the space corresponding to the slow fraction of acetylcholine. As the basement membrane surrounds the cells, it is the concentration of drugs in this layer which probably determines the concentration to which receptors are exposed. Therefore without knowing the volume of the extracellular space from which the inulin is excluded and a precise estimate of the slowly diffusing fraction, it is impossible to eliminate the possibility that the concentration to which the receptors are exposed is different from that in the bulk of the bathing solution. In addition it would be necessary to be sure that the slow fraction was indeed extracellular.

Nevertheless in view of the lack of any evidence to the contrary when considering cholinergic drugs, and there being no physical basis predicting such a partitioning effect, the normal assumption that the concentration of a drug to which the receptors are exposed is the same as that in the bathing solution, seems reasonable. However it should be noted that when considering adrenergic drugs, certain anomalous observations can be explained if the concentration of drugs to which the receptors are exposed is
not the same as that in the bulk of the bathing solution, Schild (1973).

20) The biophase model - summary

In summary therefore, the biophase model gives rise to qualitative predictions very similar to the diffusion limited model. In addition, as pointed out by Thron (1972), the qualitative predictions of a diffusion limited system will also apply to all other linear systems. Therefore the failure of potent cholinergic antagonists such as atropine to agree with these qualitative predictions means that 'no arrangement of barriers, pores, channels, pools, reservoirs, pumps, leaks or Maxwellian demons which ingenuity might suggest can explain atropine's kinetics, unless it includes some nonlinear process'.

The most likely cause for this would be the degree of binding to the receptors. Furchgott (1955) in his biophase model originally assumed that the number of receptors is so small that the binding of drug to the receptors has a negligible effect on drug distribution in the rest of the system. However Paton & Rang's (1965) subsequent studies on the degree of uptake of radioactive atropine by longitudinal muscle strips of guinea-pig ileum suggested that this assumption might not be valid and so Rang (1966) developed Furchgott's biophase model to take this into account.
The limited biophase model

In Furchgott's biophase hypothesis he assumed that the binding capacity of the receptors was negligible in relation to the amount of drug free in the biophase. Rang (1966) developed this model to take into account the binding capacity of the receptors and this version he called the limited biophase model.

If the binding capacity of the receptors is \( M \) (moles/unit weight of tissue), and the volume of the biophase is \( V \) (litres/unit weight of tissue), the binding capacity of the receptors will be \( M/V \) per unit volume of biophase. Therefore

\[
\frac{dc}{dt} = \frac{k_{in} c_a - k_{out} c_b - M d}{V dt},
\]

\( k_{in} \) and \( k_{out} \) being as defined before.

To obtain a more general solution drug concentrations and time can be expressed in dimensionless units thus:

\[
c = \frac{d}{k_{aff}} \quad \text{and} \quad t = \frac{T}{k_{out}}
\]

\( T \) is therefore the time constant for the exponential change of concentration in the biophase in the absence of appreciable receptor binding.

Substitution in equation 18 therefore gives the more general differential equation:

\[
\frac{dd_b}{dT} = \frac{k_{in} d_a - d_b - \frac{M K_{aff}}{V} d}{k_{out} dt}
\]

Rang (1966) used an analogue computer to obtain graphic solutions showing how occupancy changed with time for different values of \( M K_{aff} / V \) and for different values of \( k_{in} d_a / k_{out} \).
Similar sorts of solutions were obtained by Colquhoun & Ritchie (1972) after integrating Rang's equations.

The relevant features of these solutions are that for certain values of \( \frac{M K_{\text{aff}}}{V} \) and \( \frac{k_{\text{in}}}{k_{\text{out}}} \);

1. during the onset and offset of antagonism occupancy may change so nearly exponentially that the curvature of the log occupancy plots would not be detectable;

2. the rate of offset may appear to be independent of concentration, according to the value of \( \frac{M K_{\text{aff}}}{V} \) and the range of concentrations examined;

3. the ratio of the onset rate constant to the offset rate constant may give a reasonable estimate of the equilibrium dose ratio;

4. in addition if the value of \( \frac{M K_{\text{aff}}}{V} \) is large, the rate of interaction between fast and slow acting antagonists would be qualitatively the same as that predicted if the rate of interaction with the receptors was rate limiting.

The limited biophase model also provides an explanation for the relationship between the potency of antagonists and their rate of action. As the degree to which onset and offset is slowed is roughly proportional to the value of \( \frac{M K_{\text{aff}}}{V} \), compounds with high affinity, (i.e. more potent), will be slowed more than compounds with lower affinity.

Thron & Waud (1968) very neatly stressed the importance of \( K_{\text{aff}} \) in determining the rate at which a drug appears to act in a limited biophase system by visualizing the effect in the following way:
When a drug diffuses into a single compartment, the rate of equilibration depends on the rate of diffusion and on the volume of the space to be filled. If the compartment contains binding sites or concentrating mechanisms, these tend to increase its apparent volume, so that a longer time is required for the establishment of equilibrium.

Thus if the tissue containing $M$ moles of receptor per gram is equilibrated with a drug concentration $c$, then the receptors will take up an amount of drug equal to

$$M \frac{c}{(1 + c K_{aff})} \text{ moles per gram of tissue.}$$

This is the same quantity that would be taken up by a physical compartment of volume $M \frac{K_{aff}}{(1 + c K_{aff})}$ assuming a partition coefficient of unity.

Thus the receptors can be considered to represent a 'virtual space' equal to $M \frac{K_{aff}}{(1 + c K_{aff})}$.

Therefore when agents are compared at concentrations that produce the same degree of receptor occupancy, i.e. $cK_{aff}$ is fixed, the virtual space will be proportional to $K_{aff}$, and therefore the rate of equilibration would be expected to decrease regularly with $K_{aff}$.

The limited biophase model also provides an explanation of Thron & Waud's (1968) observation that previous treatment of longitudinal muscle strips of guinea-pig ileum with dibenamine, accelerated both the onset and offset of atropine's action, although the equilibrium constant was not significantly altered.

Using Thron & Waud's concept of 'virtual space': if the exposure to dibenamine blocked a proportion $p$ of the
receptors, when the tissue is subsequently exposed to a concentration \( c \) of atropine, the receptors will take up an amount of atropine equal to \( M \cdot c \cdot K_{aff} \cdot (1-p) / (1 + cK_{aff}) \) and so the receptors can be considered to represent a 'virtual space' equal to \( M \cdot K_{aff} \cdot (1-p) / (1 + cK_{aff}) \) which compares with the 'virtual space' if the tissue had not been previously exposed to dibenamine of \( M \cdot K_{aff} / (1 + cK_{aff}) \).

Because after previous exposure to dibenamine the 'virtual space' is less, the rate of equilibration would be expected to be faster.

Thron & Waud's (1968) observations concerning the variability of kinetic measurements could also be explained in terms of variations in \( M \), the binding capacity of the receptors per unit weight of tissue, or \( V \), the volume of the biophase per unit weight of tissue. \( M \) and \( V \) could perhaps change with the age and the sex of the animal, the season, the time between when the preparation was removed from the animal and when the experiment was conducted, the part of the ileum from which the preparation was removed, and possibly even the time after which the last 'meal' was taken.

22) The chief argument against the limited biophase model being applicable for guinea-pig ileum was expressed by Rang (1966): the predicted rate of onset and offset of occupancy is most nearly exponential for low values of \( M \cdot K_{aff} / V \), (he considered that the most appropriate value of \( M \cdot K_{aff} / V \) was 4), whereas much higher values would be necessary to explain the kinetics of interaction between slow and fast antagonists.
Tentative calculations suggest that large values of $M K_{aff} / V$ are probably the most appropriate:

Using guinea-pig ileal longitudinal muscle, Paton & Rang (1965) identified a binding site with an equilibrium constant similar to that of atropine ($K_{aff} = 0.9 \times 10^{-9} M^{-1}$) of capacity $180 \times 10^{-12}$ moles per gram wet weight of tissue. As the rate of receptor block was much faster than that corresponding to uptake at this site, the value of $M$ relevant when considering the onset of antagonism may be less than this. However this value can be used as an upper limit of $M$.

If the volume of the extracellular space is considered to be the upper limit of the value of $V$, and the inulin space of guinea-pig taenia coli is used as an estimate of this, then $V = 3.3 \times 10^{-4}$ litres per gram wet weight of tissue, Goodford & Hermansen (1961).

Therefore $\frac{M K_{aff}}{V} = \frac{(180 \times 10^{-12}) \times (0.9 \times 10^{-9})}{(3.3 \times 10^{-4})} = 490$

Therefore large values of $M K_{aff} / V$ would seem to be the most appropriate. This being the case, the evidence against the limited biophase model rests on the lack of curvature of the semi-logarithmic plots of occupancy with time during the onset and offset of antagonism. (This also applies to the kinetics of tetrodotoxin's action on the non-myelinated fibres of desheathed rabbit vagus nerves.) However even moderate curvature is difficult to detect in semilogarithmic plots unless the results are very precise and also the limited biophase model can only be considered to be a mathematical approximation of the actual physical situation. The discrepancy found between the experimentally observed relationship between occupancy
and time and that predicted by the limited biophase model could therefore be attributed to this model being only a mathematical approximation to the actual situation. If this is the case the limited biophase model could be considered to 'adequately' describe the kinetic observations.

23) Summary

The evidence that the kinetics of antagonists on guinea-pig ileum are access limited is based on the observations that are difficult to explain if interaction is rate-limiting, the fact that the kinetics of antagonists on many other tissues are not interaction limited, and the fact that the limited biophase model 'adequately' describes the kinetics of antagonists and also the anomalous observations.

Nevertheless the chief argument against access being rate-limiting is the apparent agreement between the kinetics of antagonists and the interaction-limited model.

The following study was therefore undertaken in the belief that if access was rate-limiting, under certain circumstances, the kinetics of antagonists could be shown to be inconsistent with an interaction-limited situation. This would therefore show that access determines the rate of onset and offset of antagonism despite the apparent agreement with the interaction limited model. Values of $k_1$ and $k_2$ could not therefore be determined from the rates of onset and recovery from antagonism.

It was also hoped that, even if access was found to be
rate-limiting, an experimental situation could be devised from which genuine values of $k_2$ could be determined and used to study the relationship between the structure of an antagonist and the rate at which it associates and dissociates from the receptors.
INVESTIGATIONS OF THE KINETICS OF CERTAIN COMPETITIVE ANTAGONISTS

ON GUINEA-PIG ILEUM
METHODS

A guinea-pig (weighing between 150 and 400g) was killed by a blow on the head and bled. A terminal portion of the ileum was removed and washed through with the bathing solution. From the ileum either longitudinal muscle strips or intact pieces were then prepared:

Intact lengths of ileum were prepared as described by Edinburgh Staff (1968). A 3-4cm length was suspended in the organ bath, the bottom end being held on by a glass spike and the top end being attached to the lever by a length of thread. Care was taken not to close the lumen.

Longitudinal muscle strips were prepared as described by Paton & Rang (1965): A length of washed ileum was stretched on a glass rod and the mesentery was removed. The longitudinal muscle layer was separated at one end by stroking with a cotton wool bud made on the end of blunt tweezers. By stroking in a tangential direction away from the mesenteric attachment, the muscle layer was separated around the whole circumference of the intestine and was tied with thread. By gentle tension the layer was then stripped off. A suitable length of this strip, usually with Auerbach's plexus attached along most of its length, was then suspended in the organ bath, the bottom end being attached to a glass rod by a loop of thread, and the top end being attached to the lever also by a length of thread.

The preparations were suspended in the organ bath containing either Tyrode's solution or a Krebs solution, at 36°C, and through which
either air or 95% O₂ with 5% CO₂ was bubbled, (according to whether
the bathing solution was Tyrode's or Krebs).

The Tyrode's solution was of the following composition: (mM)
149.2 Na⁺, 2.7 K⁺, 1.1 Mg²⁺, 1.8 Ca²⁺, 143.2 Cl⁻, 11.9 HCO₃⁻,
0.4 H₂PO₄⁻, 1.1 SO₄²⁻ and 1 g/litre of glucose.

The Krebs solution was of the following composition: (mM)
138 Na⁺, 5.9 K⁺, 1.2 Mg²⁺, 2.5 Ca²⁺, 122.7 Cl⁻, 25 HCO₃⁻,
1.2 H₂PO₄⁻, 1.2 SO₄²⁻ and 1 g/litre of glucose.

Unless specified otherwise, the bathing solution contained
2·76 x 10⁻⁴ M hexamethonium bromide.

The organ bath was connected to coils of glass tubing so
that the fluid in the bath could be changed by upward displacement
and overflow, either by the bathing solution or by the bathing solution
containing drugs at predetermined concentrations. The volume of the
bath was about 2·5 ml and that of the coils, 10-20 ml, so that
sufficient solution could be run through the organ bath to effect a
complete exchange (about 4 times the bath volume) without exposing
the muscle to air and without cooling.

Events in the bath were controlled by automatic apparatus
similar to that described by Schild (1946). The solutions flowed
into the organ bath at the appropriate time, determined by the
opening and closing of magnetic relays. Unless specified otherwise
the agonist was in contact with the tissue for 17 seconds. It was
then washed out twice with a 30 seconds interval between the two
washings. The next application of agonist was made 90 seconds
after the previous one.
The organ bath could be connected, via a two way stopcock, to either of two sets of 5 coils, 4 containing agonist and one without. Therefore in, say, an experiment in which the onset of antagonism was to be followed, one set of the coils could be connected to reservoirs all containing the antagonist at the required concentration, and the other set containing no antagonist. Thus by moving the key to the stopcock a clean transition could be made from one condition to the other.

The key was usually moved between the first and second washes in a cycle. In this way the tissue was first exposed to the new solutions when the second wash went into the organ bath. The cycle was usually such that the time between this moment and the moment the first wash added in the following cycle, i.e. corresponding to the peak of this first response, was one minute.

The responses produced by the agonist were recorded either with an isotonic lever with a differential transformer as a transducer, or with an isometric transducer, (Devices physiological transducer 2.S.T.0.2). With the isotonic lever a weight of between 0.5 and 0.8 g was used to load the lever when intact lengths of ileum were being used, or between 0.2 and 0.5 g when muscle strips were being used. With the isometric transducer, the tissue was set up so that it was not under an initial tension.

The voltage generated by the transducers was fed to a potentiometric pen recorder so that a visual record of the effect was obtained. The paper drive to the potentiometric recorder was switched on for only part of the cycle, from just before the agonist was added to just after it was washed out. In this way the paper could move at sufficient speed for the 'shape' of the response to be visible without generating too much paper.
The voltage signal was also linked via a digital voltmeter to an electric typewriter in such a way as to print out a number corresponding to the peak response following each application of agonist. In this way both visual (analogue), and numerical (digital) records of the effects were obtained simultaneously.

The drugs used were kindly provided by R. B. Barlow, unless specified otherwise. They were as follows:

(N.B. the abbreviations used in the text are given in brackets.)

**benziloyltropine methyliodide (BTrMa)**

![Chemical structure of benziloyltropine methyliodide (BTrMa)]

**n-pentyltriethylammonium iodide (pentyl TEA)**

![Chemical structure of n-pentyltriethylammonium iodide (pentyl TEA)]

**diphenylhydroxy acetoxyethyl dimethylethylammonium bromide (lachesine)**

![Chemical structure of diphenylhydroxy acetoxyethyl dimethylethylammonium bromide (lachesine)]

**diphenylacetoxyethyl dimethylethylammonium iodide (Ph₂ACE Me₂Et)**

![Chemical structure of diphenylacetoxyethyl dimethylethylammonium iodide (Ph₂ACE Me₂Et)]
(SY 19)

hexamethonium bromide - from Koch-Light

\[(\text{CH}_3)_3\ N^+ (\text{CH}_2)_6 - N^+ (\text{CH}_3)_3 2\text{Br}^-\]

carbaminoethylcholine chloride (carbachol) - BDH Chemicals Ltd.

\[\text{NH}_2 - \text{CO} - \text{O} - \text{CH}_2 - \text{CH}_2 - N^+ (\text{CH}_3)_3 \text{ Cl}^-\]

n-pentyltrimethylammonium iodide (pentyl TMA)

\[\text{C}_5\text{H}_{11}^+ (\text{CH}_3)_3 \text{ I}^-\]

n-hexyltrimethylammonium iodide (hexyl TMA)

\[\text{C}_6\text{H}_{13}^+ (\text{CH}_3)_3 \text{ I}^-\]

n-octyltrimethylammonium iodide (octyl TMA)

\[\text{C}_8\text{H}_{17}^+ (\text{CH}_3)_3 \text{ I}^-\]

The reference numbers given to experiments

Two sets of apparatus were available and the experiments were labelled I or II according to which apparatus had been used. In addition each guinea-pig used was given a number starting from 1. (Therefore an experiment given the reference 160 I was performed on apparatus I using ileum from guinea-pig number 160.)
Description of kinetic experiments

If the rate of change of antagonist occupancy in a given experiment is exponential this rate can be described by the corresponding time constant. For instance, for the rate of decrease in antagonist occupancy during the recovery from antagonism, the time constant can be determined by plotting antagonist occupancy corresponding to each response on a log scale against time and taking the slope of the straight line drawn through the points by eye. However this time constant does not indicate the scatter of the points. In addition it does not show how convincingly linear the relationship was.

In order to indicate the scatter of the observations and how convincingly exponential the rate of change of occupancy was, a system of graphical presentation has sometimes been used. The results of a given type of experiment were considered together and the mean occupancy at each time together with its standard error was calculated from the results of the individual experiments. These values were then plotted against time, as in DIAGRAM I.4. Although responses were obtained every 90 seconds throughout the experiments, for clarity of presentation, alternate responses are sometimes not plotted, as in this diagram. Also where the error bars would overlap the points have been displaced laterally from their true position.
PART I

THE KINETICS OF BENZILOYL-TROPINE METHYLIODIDE (BTrMe) -

Are the kinetics of this compound access limited?

In order to determine whether the kinetics of BTrMe on guinea-pig ileum are access-limited, three types of experiments were performed in which it was hoped that if access was rate-limiting, the kinetics could be shown to be inconsistent with an interaction-limited situation.

The very slow, very potent competitive antagonist BTrMe was used because deviations from the predictions of the interaction limited model would be expected to be more apparent using an antagonist as slow and as potent as BTrMe than if a faster, less potent antagonist was used. In terms of Rang's limited biophase model, the effect of the biophase is determined by the term (M K_{aff} / V) and is therefore greater when antagonists of higher affinity are used. In addition BTrMe is a quaternary compound and so it was hoped to minimize complications due to intracellular uptake.

The three types of experiments performed were as follows:

1. The rates of onset and offset of various concentrations of BTrMe were followed.

2. The decrease in BTrMe's occupancy following the superimposition of a concentration of the 'fast' antagonist n-pentyltriethyl ammonium iodide (pentyl TEA) was examined.

3. The interaction between BTrMe and pentyl TEA was also examined in conditions where the concentration of BTrMe was adjusted so that its occupancy in the absence of pentyl TEA was the same as that in equilibrium in the presence of the pentyl TEA.
In all these experiments intact pieces of ileum bathed in Tyrode's solution were used and the responses produced by carbachol were recorded using an isotonic lever. The experiments are compared with the predictions of the interaction-limited model.
I.1 THE KINETICS OF ONSET AND OFFSET OF BTrMe

Although previous workers, e.g. Paton & Rang (1965), considered that their results agreed with the predictions of the interaction-limited model, when an antagonist as potent and slow as BTrMe is used this might not be the case. The rates of onset and offset of antagonism produced by various concentrations of BTrMe were therefore followed and compared with the predictions of the interaction-limited model.

The predictions of the interaction-limited model

As shown in the introduction (p 13), if the rate of interaction between BTrMe and the receptors is the rate limiting step determining its rate of action, BTrMe’s occupancy will change during the onset and offset of antagonism in the following way:

- during onset: \[ p_t = p_{oo} (1 - \exp \left[ -\left( k_1 c + k_2 \right) t \right] ) \]
- during offset: \[ p_t = p_{oo} \exp \left[ -k_2 t \right] \]

Therefore if \( t_{on} \) is the time constant for the rate of onset of occupancy and \( t_{off} \) is the time constant for the rate of decline in occupancy,

\[ t_{on} = \frac{1}{k_1 c + k_2} \]
\[ t_{off} = \frac{1}{k_2} \]
\[ \frac{t_{off}}{t_{on}} = \frac{k_1 c}{k_2} = \frac{1}{D_{oo}} \]

Thus if interaction is rate-limiting:

1. Occupancy will change exponentially during both onset and offset
2. \( t_{off} \) will be independent of \( c \), the concentration of BTrMe
3. The ratio of the time constant for offset to the time constant for onset should be equal to the equilibrium dose ratio
Experimental Procedure

The antagonism of carbachol by BTrMe was studied using intact lengths of guinea-pig ileum suspended in Tyrode's solution. The responses were recorded by means of an isotonic lever. (details P.56)

In a few initial experiments a method was used based on the alternating technique of Edinburgh Staff (1968) for measuring the affinity constants of antagonists: Before the antagonist was added two concentrations of agonist, one double the other, were used alternately to produce contractions. Then when the antagonist was added the concentrations of agonist were adjusted to try and maintain this high-low response sequence. This was feasible for the lowest concentration of BTrMe examined and had the advantage that the difference between the high and low responses could be used as an indication of the slope of the log dose-response relationship throughout the experiment. However it was not possible to follow the faster rates and maintain the high-low sequence and so another method was used.

This method is similar to that used by Paton & Rang (1965) and is illustrated in DIAGRAM I, I, which shows an experiment in which the onset of lachesine's antagonism was followed. At the beginning of each experiment three concentrations of agonist were used in the absence of antagonist, the highest concentration (M) being double the middle (M) which was itself double the lowest (L). The concentrations were chosen so that the contractions produced by M were approximately in the middle of the dose-response curve and were repeated in the following sequence - . . . LLLL- M M M M H H H H M M M M . . .

When a stable situation had been established, usually in about 2-3 hours, the concentration M was repeated a number of times. Then
Diagram 1.1: To illustrate the method used to follow the kinetics of antagonism. (For description see text)

(The gap in the trace corresponds to a time interval of 28.5 mins)

Details
Experiment 216 I
Preparation - intact pieces of ileum
Bathing solution - Tyrode's
Lever - isotonic
Antagonist - 2 x 10^-8 M lachesine
Agonist - carbachol, concentrations (M) are indicated above the responses
there was a change to a new set of solutions to which the antagonist had been added. During onset the concentration of agonist used was doubled progressively so that the responses produced were within the control range, i.e. larger than those produced previously by L and smaller than those produced by H. The heights of the responses were thus kept as near to the M response as experimentally possible.

When equilibrium was established, three concentrations of agonist were used in the same sequence as described before. Then there was a change to a new set of solutions in which no antagonist was present and the recovery from antagonism was followed by progressively decreasing the agonist concentrations. Because recovery from this antagonist is so slow, recovery was not followed to completion.

Calculations

A log dose-response curve was taken from the last sequence of high, medium and low responses before the antagonist was added; the separate means of the last 4 low, 8 medium and 4 high responses were plotted against the log of the respective concentrations. A curve was then drawn through the three points by eye.

The dose ratio corresponding to each contraction, $DR_t$, during the onset of antagonism was then determined from this curve: If a concentration $A$ of agonist produced a response $r$ at time $t$, the concentration of agonist, $a$, which would have produced the same response in the absence of antagonist was determined from the log dose-response curve. Therefore $DR_t = A / a$.

The corresponding antagonist occupancy, $p_t$, was then calculated from $DR_t$:

$$p_t = (DR_t - 1) / (DR_t)$$

(1) & (2)
The equilibrium dose ratio, \( DR_{\infty} \), and occupancy, \( p_{\infty} \), were then calculated from the equilibrium dose response curve.

The antagonist occupancy corresponding to each response during offset was calculated in a similar way.

The calculations thus assume that the relationship between \( p \) and \( DR \) is:

\[
p = \frac{(DR - 1)}{(DR)}
\]

This assumption is examined in I.4.

**Results**

The kinetics of the antagonism produced by three concentrations of BTrMe were examined, \( 10 \times 10^{-10} M, 20 \times 10^{-10} M \) and \( 40 \times 10^{-10} M \).

The occupancy changes during onset and offset were plotted as shown in DIAGRAM I.2 using the convention of Paton & Rang (1965): for onset values of \( (p_{\infty} - p_c) \) were plotted on a log scale against time, and for offset values of \( p_c \) were plotted on a log scale against time. As there appeared to be a linear relationship between log occupancy and time, the time constants for the development and decline of antagonist occupancy were determined from the slope of the straight line drawn through the points by eye. These values are shown in TABLE I.3.

As shown in this table, \( t_{off} \) was not constant but decreased from a mean value of 398 minutes to 70 minutes when the concentration of BTrMe was increased from \( 10 \times 10^{-10} M \) to \( 40 \times 10^{-10} M \), and this was not associated with a corresponding change in the affinity constant calculated from the equilibrium dose ratio. Further, although the ratio \( t_{off}/t_{on} \) was approximately equal to the equilibrium dose ratio when the concentration of BTrMe was \( 10 \times 10^{-10} M \), when higher
DIAGRAM I.2: The kinetics of onset and offset of $10 \times 10^{-10}$ M BTrMe -
to illustrate the apparently linear relationship between occupancy
(on a log scale) and time.

For onset values of $(p_\infty^o - p_E^o)$ are plotted on the log scale against time.
For offset values of $(p_E^o)$ are plotted on the log scale against time.

Details
Experiment 39 I
Preparation - intact pieces of ileum
Bathing solution - Tyrode's
Lever - isotonic
Antagonist - $10 \times 10^{-10}$ M BTrMe (DR$\infty^o = 12$)
Agonist - carbachol
### TABLE I.3: The kinetics of onset and offset of BTrMe

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Concentration of Antagonist C</th>
<th>Time Constant for Onset</th>
<th>Time Constant for Offset</th>
<th>Equilibrium Dose Ratio</th>
<th>Antagonist Exposure Time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$t_{on}$</td>
<td>$t_{off}$</td>
<td>$t_{off}$ / $t_{on}$</td>
<td></td>
</tr>
<tr>
<td>37 I</td>
<td>$10 \times 10^{-10}$</td>
<td>24</td>
<td>532</td>
<td>22</td>
<td>16</td>
</tr>
<tr>
<td>38 I</td>
<td></td>
<td>44</td>
<td>622</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>39 I</td>
<td></td>
<td>21</td>
<td>303</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>167 I</td>
<td></td>
<td>28</td>
<td>24</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>169 II</td>
<td></td>
<td>22</td>
<td>370</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>170 II</td>
<td></td>
<td>19</td>
<td>163</td>
<td>9</td>
<td>17</td>
</tr>
<tr>
<td>Mean ± S.E.M.</td>
<td>27 ± 5</td>
<td>398 ± 82</td>
<td>16 ± 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>167 II</td>
<td>$20 \times 10^{-10}$</td>
<td>17</td>
<td>109</td>
<td>8</td>
<td>36</td>
</tr>
<tr>
<td>168 I</td>
<td></td>
<td>14</td>
<td>120</td>
<td>17</td>
<td>33</td>
</tr>
<tr>
<td>168 II</td>
<td></td>
<td>7</td>
<td>110</td>
<td>12</td>
<td>25</td>
</tr>
<tr>
<td>170 I</td>
<td></td>
<td>11</td>
<td>140</td>
<td>13</td>
<td>46</td>
</tr>
<tr>
<td>Mean ± S.E.M.</td>
<td>12 ± 2</td>
<td>120 ± 7</td>
<td>36 ± 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>171 I</td>
<td>$40 \times 10^{-10}$</td>
<td>5</td>
<td>79</td>
<td>16</td>
<td>63</td>
</tr>
<tr>
<td>171 II</td>
<td></td>
<td>5</td>
<td>61</td>
<td>12</td>
<td>61</td>
</tr>
<tr>
<td>172 II</td>
<td></td>
<td>9</td>
<td>72</td>
<td>8</td>
<td>55</td>
</tr>
<tr>
<td>Mean ± S.E.M.</td>
<td>6 ± 1</td>
<td>70 ± 5</td>
<td>60 ± 2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Details**
- **Preparation** - intact pieces of ileum
- **Bathing Solution** - Tyrode’s
- **Lever** - isotonic
- **Antagonist** - BTrMe
- **Agonist** - carbachol
concentrations of antagonist were used the difference between $t_{off}/t_{on}$ and the corresponding value of $DR_{oo}$ increased.

In view of these discrepancies between the predictions of the interaction-limited model and the observed kinetics, a closer look was taken at the way occupancy changed with time in the experiments summarized in TABLE I.3: For each concentration of BTrMe, the mean occupancy at each time was calculated from the results of the individual experiments. These mean values and their standard errors are shown in DIAGRAM I.4, again plotted using the convention of Paton & Rang (1965).

This shows that although log occupancy apparently changes linearly with time during the time in which offset was followed, during onset this is not so convincing. (The time constants given in TABLE I.3 thus refer to the initial rates.)

**Discussion**

Considering first the discrepancies between $t_{off}/t_{on}$ and the corresponding values of $DR_{oo}$; this could be accounted for by the change in $t_{off}$ as the concentration of BTrMe was increased and therefore does not necessarily provide separate evidence that the kinetics of antagonism by BTrMe is not interaction-limited.

For instance, if $t_{off}$ is taken as 398 minutes, (i.e. that corresponding to $10 \times 10^{-10} \text{ M BTrMe}$), and $t_{on}$ is taken as 12 minutes, (i.e. that for $20 \times 10^{-10} \text{ M BTrMe}$), then $t_{off}/t_{on}$ is equal to 33 which is approximately equal to the mean equilibrium dose ratio of $20 \times 10^{-10} \text{ M BTrMe}$, 36. Similarly, if $t_{off}$ is taken as 398 minutes and $t_{on}$ as 6 minutes (i.e. that for $40 \times 10^{-10} \text{ M BTrMe}$), then $t_{off}/t_{on}$ is equal to 66,
DIAGRAM I.4: The kinetics of onset and offset of BTrMe

For onset mean values of \((p_{oo} - p_c)\) ± S.E.M. are plotted on the log scale against time.

For offset mean values of \((p_c)\) ± S.E.M. are plotted on the log scale against time.

Details
(See also TABLE I.3.)

Preparation - intact pieces of ileum

Bathing solution - Tyrode's

Lever - isotonic

Antagonist - BTrMe  
10 x 10^{-10} M — O — DR_{oo} = 16 ± 1
20 x 10^{-10} M — — DR_{oo} = 30 ± 4
40 x 10^{-10} M — X — DR_{oo} = 60 ± 2

Agonist - carbachol
which is approximately equal to the mean equilibrium dose ratio of $40 \times 10^{-10} \text{M BTrMe}$, 60.

Considering now the observation that $t_{\text{off}}$ appeared to decrease as the concentration of BTrMe increased: As equilibration takes place faster following the introduction of higher concentrations of BTrMe, the slowness of offset following lower concentrations could be attributed to intracellular accumulation due to longer exposure times to the antagonist. As shown in TABLE I, there was a tendency for the antagonist exposure times to get shorter as the concentration of BTrMe was increased. However, the mean value of the exposure time in the experiments in which the kinetics of $20 \times 10^{-10} \text{M BTrMe}$ was examined, is smaller than the mean value of the $40 \times 10^{-10} \text{M}$ experiments. It therefore seems unlikely that the slowness of offset of BTrMe following the lower concentrations can be attributed to the tissue being exposed to the antagonist for a longer period.

As the smallest value of $t_{\text{off}}$ was that following $40 \times 10^{-10} \text{M BTrMe}$, $1/k_2$ could be equal to this value, i.e. the rate of recovery following $40 \times 10^{-10} \text{M BTrMe}$ could be dissociation limited although that following the lower concentrations was not. However, as the discrepancy between $t_{\text{off}}/t_{\text{on}}$ and $D_{\infty}$ is greatest in experiments in which $40 \times 10^{-10} \text{M}$ was used, the rate of onset of antagonism produced by this concentration is unlikely to have been interaction-limited if the rate of offset was.

Lastly, considering the relationship between log occupancy and time: although occupancy appeared to change exponentially with time during offset, offset was not followed until recovery was complete because of the slowness of the antagonist. It is possible that if offset had been followed for a longer period deviations would have been observed,
but it would have been impossible to distinguish genuine deviations
due to some sort of access-limitation from those due to changes in
the sensitivity of the preparation. Such changes would be
unavoidable after such a long time, over 3 hours, and would be
expected to cause an increasing deviation from linearity as $p_e$
decreased during offset.

Deviations from linearity between $\log(p_\infty - p_e)$ and time
during onset may be genuine, i.e., due to some sort of access limitation
rather than to sensitivity changes or other effects of this sort.
A deviation was apparent in the first 15 minutes of the onset of
$40 \times 10^{-10}$M despite this short time interval. Also in experiments
in which the kinetics of BTrMe were followed using longitudinal
muscle strips rather than intact ileum, (III,1), deviations from
linearity were less obvious although the actual experiments were
just as long. In addition in control experiments in which the
sensitivity of the tissue to carbachol was followed in the absence of
BTrMe over several hours, there was a tendency for the sensitivity of
the tissue to decrease which would cause the apparent rate of onset
of antagonism to increase with time. This is the opposite
of that observed during the onset of antagonism and is therefore
unlikely to have been caused by a decrease in the sensitivity of
the tissue.

Therefore the kinetics of onset and offset of BTrMe do
not appear to be consistent with the interaction limited model because:
1. $t_{off}$ decreases as the concentration of BTrMe is increased, and
2. during onset, occupancy does not appear to change exponentially
   with time.
I.2 THE INTERACTION BETWEEN BTME AND Penty1 TEA -

THE DECREASE IN BTME'S OCCUPANCY FOLLOWING THE SUPERIMPOSITION OF Penty1 TEA

Thron & Waud (1968) suggested that if access was rate limiting, the large concentration gradient when a slow antagonist is displaced from the receptors by superimposition of a fast antagonist might accelerate the removal of the slow antagonist from the tissue, as compared to simply washing out the drug. The rate of decline in BTME's occupancy on superimposition of the fast antagonist, penty1 TEA, was therefore followed and compared with that produced by lowering the concentration of BTME. The superimposition of penty1 TEA was found to accelerate the rate of decrease in BTME's occupancy and so the kinetics of offset of various concentrations of BTME, produced by superimposing various concentrations of penty1 TEA, were investigated.

Again these experiments are compared with the predictions of the interaction limited model.

The predictions of the interaction-limited model

As shown in the introduction (P 16), if the rate of interaction between BTME and the receptors is the rate-limiting step determining its rate of action, and if the fast antagonist is so fast that it can be considered to be in equilibrium at all times with the receptors not occupied by the slow antagonist, BTME's occupancy will change on superimposition of a concentration of the fast antagonist in the following way:

\[ P_t = P_{oo} - (P_{oo} - P_0) \left( \exp\left(-k_2 \left( \frac{1 + SL K_{SL} + P F_P}{1 + F K_P} \right) t \right) \right) \]  

(8)

If \( t_{off} \) is the time constant for the rate of decline in occupancy, and as \((1 + F K_P) = DR_P\) and \((1 + SL K_{SL} + F K_P) = DR_{F + SL}\), therefore:
Similarly ETM's occupancy will increase when the superimposed fast antagonist is removed in the following way:

\[ P_t = P_\infty \left(1 - \exp\left(-k_2 \left( S_{L} K_{S_{L}} + 1 \right) t \right) \right) \]  \hspace{1cm} (9)

If \( t_{on} \) is the time constant for the rate of increase in occupancy, and as \( (S_{L} K_{S_{L}} + 1) = DR_{S_{L}} \), therefore:

\[ t_{on} DR_{S_{L}} = \frac{1}{k_2} \]

In addition, if the concentration of ETM is changed from \( SL' \) to \( SL'' \), ETM's occupancy will change in the following way:

\[ P_t = P_\infty \left(1 - \exp\left(-\left( k_1 S_{L''} + k_2 \right) t \right) \right) \]  \hspace{1cm} (4)

If \( t \) is the time constant for this change in occupancy and as \( (S_{L} k_1 + k_2) = k_2 DR_{S_{L}} \), therefore:

\[ t DR_{S_{L}''} = \frac{1}{k_2} \]

Thus if interaction is rate-limiting, and the fast antagonist is so fast that it is at all times in equilibrium with the receptors not occupied by the slow antagonist:

1. ETM's occupancy will change exponentially when a concentration of the fast antagonist is superimposed, when the superimposed fast antagonist is removed, and when the concentration of ETM is changed from one level to another.

2. \( t_{off} \frac{DR_{F+SL}}{DR_{F'}} \) should be equal to \( t_{on} DR_{S_{L}} \) and also \( t DR_{S_{L}''} \).

Experimental Procedure

As before, carbachol contractions of intact pieces of ileum
in Tyrode's solution were recorded isotonically. (details p.56)

The method used was similar to that of 1,1 and is illustrated in Diagram 1.5, which shows an experiment in which the fast antagonist pentyl TEA was superimposed on the slow antagonist lachesine. At the beginning of each experiment three concentrations of agonist were used in the presence of the slow antagonist until a stable situation had been established. Then there was a change to a new set of solutions containing both the slow and the fast antagonist.

The establishment of the new equilibrium was then followed by progressively decreasing the carbachol concentrations from an initially increased level.

In a few experiments the change in dose ratio was followed to its new equilibrium level. The fast antagonist was then removed, by changing to a new set of solutions in which only the slow antagonist was present, and the re-establishment of equilibrium followed by progressively increasing the carbachol concentration from an initially decreased level.

However in most cases the change in dose ratio following the superimposition of the fast antagonist was not followed to equilibrium, because of the amount of pentyl TEA which would have been required to follow all the experiments to an equilibrium. In these experiments therefore the re-establishment of equilibrium on removal of the fast antagonist was not followed.

In addition in a few experiments the decrease or increase of BTrMe's occupancy produced by a change in the concentration of BTrMe was followed by progressively adjusting the carbachol concentration as described previously.
DIAGRAM I: To illustrate the method used to follow the interaction between a fast antagonist (in this case pentyl TEA) and a slow antagonist (in this case lachesine). (For description see text)

Details

Experiment 207 I
Preparation - intact pieces of ileum
Bathing solution - Tyrode's
Lever - isotonic
Antagonist - $0.5 \times 10^{-8} \text{ M lachesine}$
$25 \times 10^{-4} \text{ M pentyl TEA}$
Agonist - carbachol, concentrations (M) are indicated above the responses
Calculations

A log dose-response curve was taken from the last sequence of high, medium, and low responses before the fast antagonist was added or the concentration of slow changed; the separate means of the last 4 low, 8 medium and 4 high responses were plotted against the log of the respective concentrations. A curve was then drawn through the points by eye.

The slow antagonist's occupancy corresponding to each response after the superimposition of the fast antagonist was then calculated as follows:

The dose ratio, $\text{DR}_{F+SL,t}$, corresponding to each contraction was determined from the log dose-response relationship: If a concentration $A_{SL+F}$ of agonist at time $t$ in the presence of the slow and the fast antagonist produced a response $r$, the concentration, $A_{SL}$, which would have produced the same response in the presence of the slow antagonist only was determined from the log dose-response curve.

If $a$ is the concentration of agonist which would have been required to produce the same response in the absence of antagonist,

$$\frac{A_{SL+F}}{a} = \text{DR}_{SL+F,t}$$

and

$$A_{SL} = \text{DR}_{SL}$$

Thus

$$\frac{A_{SL+F}}{A_{SL}} = \frac{\text{DR}_{SL+F,t}}{\text{DR}_{SL}}$$

$\text{DR}_{F+SL,t}$ was therefore calculated from the ratio $A_{SL+F}/A_{SL}$ and $\text{DR}_{SL}$, calculated by assuming that $K_{SL} = 1.5 \times 10^{10} \text{L.mol}^{-1}$, (see 1.4).

The occupancy of the slow antagonist corresponding to each contraction, $p_{SL,t}$, was then calculated from the combined dose ratio, $\text{DR}_{SL+F,t}$: If the fast antagonist is so fast that it can be considered to be at equilibrium at all times with the receptors not occupied by
the slow antagonist, then the proportion of receptors occupied by the 
fast antagonist, \( p_F \), is given by:

\[
p_F = \frac{F K_F (1 - P_{SL})}{1 + F K_F}
\]

Therefore

\[
(P_{SL} + P_F) = \frac{P_{SL} + F K_F}{1 + F K_F}
\]

But

\[
DR_{F+SL} = \frac{1}{1 - (P_{SL} + P_F)}
\]

Therefore

\[
P_{SL,t} = \frac{DR_{F+SL,t} - DR_F}{DR_{F+SL,t}}
\]

\( P_{SL,t} \) was therefore calculated from the combined dose ratio \( DR_{F+SL,t} \),
and \( DR_F \) calculated by assuming that \( DR_F = 1 + F K_F \) and \( K_F = 3.6 \times 10^4 M^{-1} \),
(see I.4).

The slow antagonist's occupancy corresponding to each response
after removal of the superimposed fast antagonist was calculated
as follows:

The dose ratio \( DR_t \) corresponding to each contraction was
again determined from the log dose-response curve: if a concentration
\( A \) of agonist at time \( t \) after removal of the superimposed fast antagonist
produced a response \( r \), the concentration \( A_{SL} \) which would have produced
the same response when the tissue was in equilibrium with the slow
antagonist was determined from the log dose-response curve.

If \( a \) is the concentration of agonist which would have
been required to produce the same response in the absence of antagonist,
then,

\[
\frac{A_{SL}}{a} = DR_{SL} \quad \text{and} \quad \frac{A}{a} = DR_t
\]

Therefore,

\[
\frac{A}{\frac{A_{SL}}{DR_{SL}}} = \frac{DR_t}{DR_{SL}}
\]

\( DR_t \) was therefore calculated from the ratio \( A/A_{SL} \) and \( DR_{SL} \),
calculated by assuming that $DR_{SL} = 1 + SL K_{SL}^*$ and $K_{SL}^* = 1.5 \times 10^{10} M^{-1}$, see I.4.

If it is assumed that the fast antagonist is so fast that its occupancy immediately falls to zero when it is removed, the dose ratio $DR_t$ corresponding to each contraction after the fast antagonist was removed must be due to the occupancy of the slow antagonist. The occupancy of the slow antagonist corresponding to each contraction, $p_{SL,t}$ would then be equal to $(DR_t - 1)/(DR_t)$. Values of $p_{SL,t}$ were calculated in this way.

The slow antagonist's occupancy corresponding to each response after changing the concentration from SL' to SL'' was calculated as follows:

The dose ratio corresponding to each response, $DR_t$ was determined from the log dose-response relationship as before, assuming that the equilibrium dose ratio produced by SL' was equal to $(1 + SL' K_{SL}^*)$, $K_{SL}^*$ being $1.5 \times 10^{10} M^{-1}$. Therefore:

$$p_{SL,t} = \frac{DR_t - 1}{DR_t}$$

The calculations in this section thus assume that:

- $DR_{SL} = 1 + SL K_{SL}^*$, $K_{SL}^*$ being equal to $1.5 \times 10^{10} M^{-1}$
- $DR_F = 1 + F K_F^*$, $K_F^*$ being equal to $3.6 \times 10^4 M^{-1}$
- $DR_F + SL = DR_F + DR_{SL} - 1$
- $p_F = \frac{F K_F^* (1 - p_{SL})}{1 + F K_F}$

These assumptions are examined in I.4.
Results

The kinetics of offset and onset of BTrMa's occupancy produced by changing the concentration of BTrMa and the kinetics of offset and onset produced by superimposing (or removing the superimposed) pentyl TEA

The occupancy changes during onset and offset were plotted as shown in DIAGRAM 1.6. As before, for onset, values of \((p_{oo} - p_c)\) were plotted on a log scale against time, and for offset, values of \((p_c - p_{oo})\) were plotted on a log scale against time.

As predicted by the interaction limited model there appeared to be a linear relationship between log occupancy and time during the offset of occupancy and onset following an increase in BTrMa's concentration. However the rate of onset following the removal of the superimposed pentyl TEA was found to be exponential only when low concentrations of pentyl TEA were used, \(P \leq \text{2.} \times \text{10}^{-4} \text{M}\). This may be because, when the concentration of pentyl TEA is greater than this, the occupancy of pentyl TEA does not immediately fall to zero when the fast antagonist is removed. (see I.4)

When occupancy was found to change exponentially with time, the time constant for this change was determined from the slope of the straight line drawn through the points by eye. TABLE I.7 shows the results of these experiments.

Contrary to the predictions of the interaction-limited model \(t_{OFF}^{\text{DR}_P + \text{SL}/\text{DR}_F}\) was found to be smaller than the values of \(t_{ON}^{\text{DR}_{SL}}\); and also the values of \(t_{OFF}^{\text{DR}_{SL}}\).

The relationship between \(t_{OFF}^{\text{DR}_{P+SL}/\text{DR}_F}\) and the concentrations of the two antagonists

The relationship between \(t_{OFF}^{\text{DR}_{P+SL}/\text{DR}_F}\) and the concentrations
DIAGRAM I.6: The rate of decrease of BTrMe's occupancy on superimposing pentyl TEA

Values of $(p_t - p_\infty)$ are plotted on the log scale against time

Details

Experiment 121 I
Preparation - intact pieces of ileum
Bathing Solution - Tyrode's
Lever - isotonic
Antagonists - BTrMe $20 \times 10^{-10} \text{M}$
pentyl TEA $2.5 \times 10^{-4} \text{M}$
Agonist - carbachol
TABLE I.7: The kinetics of offset and onset of BTrMe's occupancy produced by either changing the concentration of BTrMe or superimposing, (or removing the superimposed), pentyl TEA

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Initial Conc. of BTrMe</th>
<th>Conc. of Pentyl TEA</th>
<th>Changing the Conc. of BTrMe</th>
<th>Interaction with pentyl TEA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Offset</td>
</tr>
<tr>
<td></td>
<td>SL'</td>
<td>p SL' = p SL''</td>
<td></td>
<td>t off_{DR_{SL'}}</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>M</td>
<td></td>
<td>mins</td>
</tr>
<tr>
<td>109 I</td>
<td>20 x 10^{-10}</td>
<td>0.97 - 0.61</td>
<td></td>
<td>187</td>
</tr>
<tr>
<td>109 II</td>
<td></td>
<td></td>
<td></td>
<td>140</td>
</tr>
<tr>
<td>Mean ± S.E.M.</td>
<td></td>
<td></td>
<td></td>
<td>164 ± 24</td>
</tr>
<tr>
<td>111 I</td>
<td>20 x 10^{-10}</td>
<td>0.97 - 0.76</td>
<td>2.5 x 10^{-4}</td>
<td>201</td>
</tr>
<tr>
<td>112 I</td>
<td></td>
<td></td>
<td></td>
<td>157</td>
</tr>
<tr>
<td>Mean ± S.E.M.</td>
<td></td>
<td></td>
<td></td>
<td>179 ± 22</td>
</tr>
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<td>104 I</td>
<td>60 x 10^{-10}</td>
<td>0.99 - 0.95</td>
<td>2.5 x 10^{-4}</td>
<td>65</td>
</tr>
<tr>
<td>110 I</td>
<td></td>
<td></td>
<td></td>
<td>101</td>
</tr>
<tr>
<td>113 I</td>
<td></td>
<td></td>
<td></td>
<td>180</td>
</tr>
<tr>
<td>114 II</td>
<td></td>
<td></td>
<td></td>
<td>46</td>
</tr>
<tr>
<td>Mean ± S.E.M.</td>
<td></td>
<td></td>
<td></td>
<td>115 ± 34</td>
</tr>
</tbody>
</table>

SL' is the initial concentration of BTrMe. DR_{SL'} is the equilibrium dose ratio, and p_{SL'} the equilibrium occupancy, produced by this concentration. SL'' is less than SL'. DR_{SL''} is the equilibrium dose ratio and p_{SL''} the equilibrium occupancy produced by this concentration.

p_{SL''} is also the occupancy of BTrMe at equilibrium following the superimposition of a concentration F of pentyl TEA on a concentration SL' of BTrMe.

$$DR_{SL' + F} = \frac{A_{SL' + F}}{A_{SL'}}$$ where $A_F = 3.6 \times 10^4 M^{-1}$

Details
Preparation - intact pieces of ileum
Bathing Solution - Tyrode's
Lever - isotonic
Antagonists - BTrMe & pentyl TEA
Agonist - carbachol
of the slow and the fast antagonist was therefore investigated. Concentrations of BTrMe between $6 \times 10^{-10}$M and $40 \times 10^{-10}$M, and concentrations of pentyl TEA between $1.3 \times 10^{-4}$M and $45 \times 10^{-4}$M, were examined. In all these experiments there appeared to be an exponential relationship between BTrMe's occupancy and time, as predicted by the interaction-limited model. This is illustrated in Diagram I.8. Tables I.9 summarize the results of these experiments.

As shown in Diagram I.10, as the concentration of pentyl TEA was increased the resulting $t_{\text{off}}$ of $DR_{T+SL}/DR_{T}$ decreases to what appears to be a limiting value of about 20 minutes.

Diagram I.11 shows the relationship between $t_{\text{off}}$ of $DR_{T+SL}/DR_{T}$ and the change in occupancy, $(P_{SL} - P_{SL}^*)$. As the change in occupancy was increased the resulting $t_{\text{off}}$ of $DR_{T+SL}/DR_{T}$ also decreased but this time was dependent on the concentration of BTrMe.

Discussion

Apparent values of $k_2$ can be calculated from the offset time constants, $t_{\text{off}}$, using the equations given on P.71. As the true value of $1/k_2$ for BTrMe must be equal or less than the lowest value determined experimentally from the kinetics of antagonism, rates which correspond to values greater than this can not be interaction limited.

Therefore as the initial experiments, Table I.7, showed that $t_{\text{off}}$ of $DR_{T+SL}/DR_{T}$ was smaller than $t_{\text{on}}$ of $DR_{SL}$ and $t_{\text{DR}_{SL}^{*}}$, the rates of onset and offset when the concentration of BTrMe is changed, and the rate of onset following the removal of the superimposed pentyl TEA can not be interaction limited.

In addition the subsequent experiments, Table I.9, showed that
Mean values of \((p_L - p_{\infty})\) are plotted on a log scale against time.

Details:
(See also TABLE I.9)
Preparation - intact pieces of ileum
Bathing solution - Tyrode's
Lever - isotonic
Antagonists - 40 x 10^{-10} M BTrMe
Pentyl TEA, concentrations as indicated
Agonist - carbachol
TABLE 1.9 (a): The kinetics of offset of BTrMe's occupancy produced by superimposing various concentrations of pentyl TEA - summary

<table>
<thead>
<tr>
<th>Concentration of BTrMe</th>
<th>pSL' - pSL&quot;</th>
<th>Concentration of pentyl TEA F</th>
<th>DR_F+SL M</th>
<th>t_off (mins)</th>
<th>Mean ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>SL' = 6 x 10^-10</td>
<td>.91 - .52</td>
<td>2.2 x 10^-4</td>
<td>2.2 x 10^-4</td>
<td>136.6 ± 23.4</td>
<td>(5)</td>
</tr>
<tr>
<td></td>
<td>.91 - .49</td>
<td>2.5 x 10^-4</td>
<td>2.5 x 10^-4</td>
<td>100.0 ± 26.7</td>
<td>(4)</td>
</tr>
<tr>
<td></td>
<td>.91 - .32</td>
<td>5.4 x 10^-4</td>
<td>5.4 x 10^-4</td>
<td>95.5 ± 17.2</td>
<td>(4)</td>
</tr>
<tr>
<td></td>
<td>.91 - .20</td>
<td>10.3 x 10^-4</td>
<td>10.3 x 10^-4</td>
<td>63.0 ± 8.4</td>
<td>(4)</td>
</tr>
<tr>
<td></td>
<td>.91 - .10</td>
<td>25.0 x 10^-4</td>
<td>25.0 x 10^-4</td>
<td>33.2 ± 6.7</td>
<td>(4)</td>
</tr>
<tr>
<td>SL' = 10 x 10^-10</td>
<td>.94 - .74</td>
<td>1.3 x 10^-4</td>
<td>1.3 x 10^-4</td>
<td>148.5 ± 22.0</td>
<td>(4)</td>
</tr>
<tr>
<td></td>
<td>.94 - .61</td>
<td>2.5 x 10^-4</td>
<td>2.5 x 10^-4</td>
<td>124.5 ± 21.3</td>
<td>(4)</td>
</tr>
<tr>
<td></td>
<td>.94 - .55</td>
<td>3.3 x 10^-4</td>
<td>3.3 x 10^-4</td>
<td>132.4 ± 18.8</td>
<td>(4)</td>
</tr>
<tr>
<td></td>
<td>.94 - .42</td>
<td>6.0 x 10^-4</td>
<td>6.0 x 10^-4</td>
<td>119.3 ± 16.3</td>
<td>(4)</td>
</tr>
<tr>
<td></td>
<td>.94 - .35</td>
<td>7.8 x 10^-4</td>
<td>7.8 x 10^-4</td>
<td>85.3 ± 8.4</td>
<td>(4)</td>
</tr>
<tr>
<td></td>
<td>.94 - .24</td>
<td>14.0 x 10^-4</td>
<td>14.0 x 10^-4</td>
<td>48.7 ± 5.8</td>
<td>(4)</td>
</tr>
<tr>
<td></td>
<td>.94 - .15</td>
<td>25.0 x 10^-4</td>
<td>25.0 x 10^-4</td>
<td>30.5 ± 7.8</td>
<td>(4)</td>
</tr>
<tr>
<td>SL' = 20 x 10^-10</td>
<td>.97 - .76</td>
<td>2.5 x 10^-4</td>
<td>2.5 x 10^-4</td>
<td>84.2 ± 8.4</td>
<td>(11)</td>
</tr>
<tr>
<td></td>
<td>.97 - .58</td>
<td>6.0 x 10^-4</td>
<td>6.0 x 10^-4</td>
<td>113.7 ± 16.7</td>
<td>(9)</td>
</tr>
<tr>
<td></td>
<td>.97 - .38</td>
<td>14.0 x 10^-4</td>
<td>14.0 x 10^-4</td>
<td>48.0 ± 5.5</td>
<td>(4)</td>
</tr>
<tr>
<td></td>
<td>.97 - .26</td>
<td>25.0 x 10^-4</td>
<td>25.0 x 10^-4</td>
<td>43.3 ± 7.2</td>
<td>(8)</td>
</tr>
<tr>
<td>SL' = 40 x 10^-10</td>
<td>.99 - .76</td>
<td>4.8 x 10^-4</td>
<td>4.8 x 10^-4</td>
<td>78.4 ± 19.7</td>
<td>(5)</td>
</tr>
<tr>
<td></td>
<td>.99 - .60</td>
<td>11.5 x 10^-4</td>
<td>11.5 x 10^-4</td>
<td>53.0 ± 8.1</td>
<td>(5)</td>
</tr>
<tr>
<td></td>
<td>.99 - .40</td>
<td>26.5 x 10^-4</td>
<td>26.5 x 10^-4</td>
<td>29.8 ± 4.5</td>
<td>(5)</td>
</tr>
<tr>
<td></td>
<td>.99 - .33</td>
<td>35.0 x 10^-4</td>
<td>35.0 x 10^-4</td>
<td>23.8 ± 3.8</td>
<td>(4)</td>
</tr>
<tr>
<td></td>
<td>.99 - .28</td>
<td>45.0 x 10^-4</td>
<td>45.0 x 10^-4</td>
<td>21.3 ± 0.9</td>
<td>(3)</td>
</tr>
</tbody>
</table>

( ) Number of estimations made, each using ileum from a different guinea-pig

SL' is the initial concentration of BTrMe; pSL' is the equilibrium occupancy produced by this concentration = SL'KSL/(1 + SI'KSL)

F is the concentration of the fast antagonist superimposed

pSL" is the occupancy of BTrMe when at equilibrium with the fast antagonist = SL'KSL/(1 + SL'KSL + F K_F)

DR_F+SL = 1 + F K_F + SL K_SL, and DR_F = 1 + F K_F

K_SL = 1.5 x 10^10 M^-1, K_F = 3.6 x 10^4 M^-1

Details
Preparation - intact pieces of ileum
Bathing solution - Tyrode's
Lever - isotonic
Antagonists - BTrMe
Agonist - carbachol
TABLE I.9. (b): The kinetics of offset of BTrMe's occupancy produced by superimposing various concentrations of pentyl TEA - the rates observed in the individual experiments

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Concentration of BTrMe ( \text{SL} ) ( \text{M} )</th>
<th>Concentration of pentyl TEA ( F ) ( \text{M} )</th>
<th>( t_{\text{off}} ) ( \text{DR}_{F+SL} ) (mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>160 II</td>
<td>( 6 \times 10^{-10} )</td>
<td>( 2.2 \times 10^{-4} )</td>
<td>120</td>
</tr>
<tr>
<td>158 I</td>
<td>( 6 \times 10^{-10} )</td>
<td>( 2.5 \times 10^{-4} )</td>
<td>79</td>
</tr>
<tr>
<td>152 II</td>
<td>( 6 \times 10^{-10} )</td>
<td>( 5.4 \times 10^{-4} )</td>
<td>76</td>
</tr>
<tr>
<td>145 II</td>
<td>( 6 \times 10^{-10} )</td>
<td>( 10.3 \times 10^{-4} )</td>
<td>57</td>
</tr>
<tr>
<td>143 II</td>
<td>( 6 \times 10^{-10} )</td>
<td>( 25 \times 10^{-4} )</td>
<td>37</td>
</tr>
<tr>
<td>158 II</td>
<td>( 10 \times 10^{-10} )</td>
<td>( 1.3 \times 10^{-4} )</td>
<td>205</td>
</tr>
<tr>
<td>155 I</td>
<td>( 10 \times 10^{-10} )</td>
<td>( 2.5 \times 10^{-4} )</td>
<td>102</td>
</tr>
<tr>
<td>149 I</td>
<td>( 10 \times 10^{-10} )</td>
<td>( 3.3 \times 10^{-4} )</td>
<td>120</td>
</tr>
<tr>
<td>140 I</td>
<td>( 10 \times 10^{-10} )</td>
<td>( 2.5 \times 10^{-4} )</td>
<td>120</td>
</tr>
<tr>
<td>155 II</td>
<td>( 10 \times 10^{-10} )</td>
<td>( 3.3 \times 10^{-4} )</td>
<td>120</td>
</tr>
<tr>
<td>153 I</td>
<td>( 10 \times 10^{-10} )</td>
<td>( 3.3 \times 10^{-4} )</td>
<td>120</td>
</tr>
<tr>
<td>144 I</td>
<td>( 10 \times 10^{-10} )</td>
<td>( 6 \times 10^{-4} )</td>
<td>97</td>
</tr>
<tr>
<td>142 I</td>
<td>( 10 \times 10^{-10} )</td>
<td>( 6 \times 10^{-4} )</td>
<td>97</td>
</tr>
<tr>
<td>138 I</td>
<td>( 10 \times 10^{-10} )</td>
<td>( 7.8 \times 10^{-4} )</td>
<td>72</td>
</tr>
<tr>
<td>135 I</td>
<td>( 10 \times 10^{-10} )</td>
<td>( 7.8 \times 10^{-4} )</td>
<td>72</td>
</tr>
<tr>
<td>140 II</td>
<td>( 10 \times 10^{-10} )</td>
<td>( 7.8 \times 10^{-4} )</td>
<td>72</td>
</tr>
<tr>
<td>150 I</td>
<td>( 10 \times 10^{-10} )</td>
<td>( 7.8 \times 10^{-4} )</td>
<td>72</td>
</tr>
<tr>
<td>156 II</td>
<td>( 10 \times 10^{-10} )</td>
<td>( 7.8 \times 10^{-4} )</td>
<td>72</td>
</tr>
</tbody>
</table>

contd/
<table>
<thead>
<tr>
<th>SL'</th>
<th>F</th>
<th>$t_{off}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>115 I</td>
<td>$10 \times 10^{-10}$</td>
<td>14 $\times 10^{-4}$</td>
</tr>
<tr>
<td>116 II</td>
<td>$10 \times 10^{-10}$</td>
<td>25 $\times 10^{-4}$</td>
</tr>
<tr>
<td>118 I</td>
<td>20 $\times 10^{-10}$</td>
<td>2.5 $\times 10^{-4}$</td>
</tr>
<tr>
<td>121 II</td>
<td>20 $\times 10^{-10}$</td>
<td>6 $\times 10^{-4}$</td>
</tr>
<tr>
<td>126 II</td>
<td>20 $\times 10^{-10}$</td>
<td>14 $\times 10^{-4}$</td>
</tr>
<tr>
<td>134 I</td>
<td>20 $\times 10^{-10}$</td>
<td>25 $\times 10^{-4}$</td>
</tr>
<tr>
<td>139 I</td>
<td>40 $\times 10^{-10}$</td>
<td>4.8 $\times 10^{-4}$</td>
</tr>
<tr>
<td>145 I</td>
<td>40 $\times 10^{-10}$</td>
<td>11.5 $\times 10^{-4}$</td>
</tr>
<tr>
<td>138 II</td>
<td>40 $\times 10^{-10}$</td>
<td>11.5 $\times 10^{-4}$</td>
</tr>
<tr>
<td>SL</td>
<td>F</td>
<td>t_{off}</td>
</tr>
<tr>
<td>----</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>161 II</td>
<td>40 x 10^{-10}</td>
<td>26.5 x 10^{-4}</td>
</tr>
<tr>
<td>153 II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>146 II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>141 II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>135 II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>165 I</td>
<td>40 x 10^{-10}</td>
<td>35 x 10^{-4}</td>
</tr>
<tr>
<td>165 II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>166 I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>166 II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>163 II</td>
<td>40 x 10^{-10}</td>
<td>45 x 10^{-4}</td>
</tr>
<tr>
<td>164 I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>164 II</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The relationship between $t_{\text{off}} \frac{D_{P+S+L}}{D_{P}}$ and $F$. If the rate of interaction with the receptors is rate-limiting $t_{\text{off}} \frac{D_{P+S+L}}{D_{P}} = 1/k_2$

$t_{\text{off}}$ is the time constant for the decrease in $B_{TrMe}$'s occupancy on superimposition of pentyl TEA

$D_{P+S+L} = 1 + F K_F + SL K_{SL}$, and $D_{P} = 1 + F K_P$,  $(K_F = 3.6 \times 10^{-4} M^{-1}, K_{SL} = 1.5 \times 10^{-10} M^{-1})$

Details
(See also TABLE I.9)
Preparation - intact pieces of ileum
Bathing solution - Tyrode's
Lever - isotonic
Antagonists-$B_{TrMe}$ $6 \times 10^{-10} M \bigtriangleup$
$10 \times 10^{-10} M \bigtriangleup$
$20 \times 10^{-10} M \bigcirc$
$40 \times 10^{-10} M \bigcirc$
-pentyl TEA

Agonist - carbachol
The relationship between $t_{\text{off}}\frac{\Delta R_{Tf} SL}{\Delta R_{Tf}}$ and the change in BTrMe's occupancy, $(p_{SL} - p_{SL'}^*)$, produced by superimposition of Pentyl TEA is the time constant for the decrease in BTrMe's occupancy.

$DR_{Tf} SL = 1 + F K_f S L K_{SL}$, and $DR_{Tf} = 1 + F K_f$, ($K_f = 3.6 \times 10^{-4} \text{ M}^{-1}$, $K_{SL} = 1.5 \times 10^{-10} \text{ M}^{-1}$)

Details
(See also TABLE I.9 and DIAGRAM I.10)
Preparation - intact pieces of ileum
Bathing solution - Tyrode's
Lever - isotonic

Antagonists - BTrMe $6 \times 10^{-10} \text{ M} \times$
$10 \times 10^{-10} \text{ M} \triangle$
$20 \times 10^{-10} \text{ M} \bigcirc$
$40 \times 10^{-10} \text{ M} \bigodot$

- pentyl TEA
$t_{off} \frac{DR_{T+SL}}{DR_{F}}$ decreased to a limiting value of around 20 minutes, so rates of offset corresponding to values greater than this cannot be interaction limited.

Therefore the only possible situation where interaction could be rate limiting is following the superimposition of very large concentrations of pentyl TEA. If this were the case $k_2$ would be approximately equal to $1/20 \text{ mins}^{-1}$. However the possibility can not be ruled out that some different access limitation is now operating. This possibility is considered further in section III.
1.3 THE INTERACTION BETWEEN BTrMe AND PentyI TEA WHEN THE CONCENTRATION OF BTrMe IS ADJUSTED TO MAINTAIN ITS EQUILIBRIUM OCCUPANCY

In addition to the experiments described in 1.2, the interaction between BTrMe and pentyl TEA was also investigated in another way: As in 1.2, the tissue was initially equilibrated with a concentration of the slow antagonist, but when the fast antagonist was superimposed, the concentration of the slow antagonist was simultaneously increased so that its occupancy at the new equilibrium in the presence of the fast was the same as before. Therefore if interaction is rate-limiting, and if the fast antagonist is sufficiently fast, BTrMe's occupancy should be maintained.

Theory

If the initial concentration of BTrMe was \( SL^1 \) and that used in the presence of the pentyl TEA was \( SL^2 \), the equilibrium occupancy of the BTrMe in the two conditions will be the same if \( SL^2 = SL^1 (1 + F K_p) \):

The proportion of the receptors occupied by BTrMe in a concentration \( SL^1 \) is:

\[
P_{SL^1} = \frac{SL^1 K_{SL}}{1 + SL^1 K_{SL}}
\]

and the proportion of the receptors occupied by BTrMe in a concentration \( SL^2 \) in the presence of a concentration \( F \) of pentyl TEA is:

\[
P_{SL^2} = \frac{SL^2 K_{SL}}{1 + F K_p + SL^2 K_{SL}}
\]

Therefore if \( SL^2 = SL^1 (1 + F K_p) \):

\[
P_{SL^2} = \frac{SL^1 K_{SL} (1 + F K_p)}{(1 + F K_p) + SL^1 K_{SL} (1 + F K_p)}
\]

\[
= \frac{SL^1 K_{SL}}{1 + SL^1 K_{SL}}
\]

\[
= P_{SL^1}
\]
Thus if the receptors are in equilibrium with a concentration \( SL' \) of the slow antagonist and a concentration \( F \) of a fast antagonist is superimposed, and if at the same time the concentration of the slow antagonist is increased to \( SL'' \), where \( SL'' = SL'I + F \), the new equilibrium occupancy of the slow antagonist should be the same as before.

Further if the rate of interaction with the receptors is the rate-limiting step, the concentrations of antagonist(s) in the proximity of the receptors will rise immediately from \( SL' \) to \( SL'' \) and \( F \). Thus if the speed of the fast antagonist is such that it equilibrates immediately with the receptors not occupied by the slow antagonist, the dose ratio should change immediately from \( DR_{SL'} \) to \( DR_{SL'' + F} \) with no transitional stage.

However if access is rate-limiting, the concentration of fast in the proximity of the receptors will rise quickly to \( F \), whereas the concentration of slow will rise only slowly from \( SL' \) to \( SL'' \). Because of this lag, the fast antagonist would initially 'displace' the slow antagonist from the receptors, the occupancy of the slow antagonist being subsequently restored as the concentration of the slow antagonist in the proximity of the receptors rises to \( SL'' \).

**Procedure**

As before, carbachol contractions of intact pieces of ileum in Tyrode's solution were recorded isotonically, (details p.56).

The method used was similar to that used previously and is illustrated in DIAGRAM I.12: The tissue was set up in the presence of a certain concentration of the slow antagonist, \( SL' \), three concentrations of agonist being used. When a stable situation was
Diagram I.12: The interaction between BTrMe and pentyl TEA
(The gap in the trace corresponds to a time interval of 57 mins.)

Details
Experiment 195 II
Preparation - intact pieces of ileum
Bathing solution - Tyrode's
Lever - isotonic
Antagonists - BTrMe & pentyl TEA
Agonist - carbachol, concentrations (M) indicated above responses
established a concentration of pentyl TEA was superimposed and at the same time the concentration of BTrMe was increased to SL". The establishment of this equilibrium was followed with a single concentration of carbachol. The pentyl TEA was then removed and the concentration of the slow antagonist decreased to SL'. The re-establishment of equilibrium was again followed with a single concentration of carbachol.

Calculation

The occupancy of BTrMe corresponding to each response after the addition of the fast antagonist was calculated as in I.2.

As the occupancy of BTrMe corresponding to the first response was less than \( p_{SL_1} \), the time constant for the decrease in BTrMe's occupancy was calculated by assuming that the decrease was exponential between when the solutions were changed and the first response. The change over to the new solutions was made so that the peak of the first response was one minute after the change. Therefore:

\[
t_{off} = \frac{\log p_{SL_1} - \log(p_{SL}/e)}{\log p_{SL_1} - \log p}
\]

where \( p \) is the occupancy of BTrMe corresponding to the first response.

The occupancy of BTrMe corresponding to each response after the removal of the fast antagonist was also calculated as in I.2.

The assumptions involved in this section are similar to those of I.2. In particular it is assumed that if \( SL'^1 = SL^1 (1 + F K_F) \) and \( K_F = 3.6 \times 10^4 M^{-1} \), then \( p_{SL_1} = p_{SL''} \). These assumptions are examined in I.4.
Results

When the concentration of the fast antagonist was sufficiently large a transitional stage was observed as shown in DIAGRAM 1.12. BTrMe's occupancy corresponding to the first contraction was less than $p_{SL}^1$, and as $F$ was increased this difference increased.

The subsequent restoration of BTrMe's occupancy is illustrated in DIAGRAM 1.13. Although this shows the wide scatter of the values of $p_e$ an exponential relationship between $p_e$ and time can not be excluded and so values of $t_{on}$ were determined for each experiment.

However when the fast antagonist was removed there did not appear to be an exponential relationship between $p_{SL}^1$ and time, probably because $p_F$ does not immediately fall to zero, see 1.4.

TABLE I.14 shows the various combinations of SL$^1$ and F used, together with the time constant for the offset of BTrMe's occupancy during the first minute, $t_{off}$, multiplied by $DR_{F+SL}/DR_F$, and the time constant for the subsequent re-establishment of BTrMe's occupancy, $t_{on}$.
The rate of restoration of BTrMa's occupancy in 3-10x10^-6 M experiments was initially equilibrated with 10x10^-6 M pentyl TEA, after which the concentration of BTrMa was increased to 5x10^-6 M. This appeared to cause an initial displacement of BTrMa's occupancy followed by its subsequent restoration, as shown in this diagram. Mean values of (P_p - P_T) ± S.E.M. are plotted on a log scale against time. (When pentyl TEA was superimposed, t=0).

Details (see also Table 1.14) Preparation - intact pieces of ileum Bathing solution - Tyrode's Iso-osmotic Agonist - carbachol
The tissue was initially equilibrated with a concentration SL' of BTrMe; then the concentration of BTrMe was increased to SL" and a concentration, F, of pentyl TEA was added. This appeared to cause an initial displacement of BTrMe's occupancy followed by its subsequent restoration.

t_{off} is the time constant corresponding to the initial displacement

t_{on} is the time constant for the subsequent restoration of occupancy

\[
\frac{\text{DR}_{\text{SL}'+F}}{\text{DR}_{\text{SL}''}} = 1 + \text{SL}'' K_{\text{SL}'} F K_F, \quad \text{and} \quad \frac{\text{DR}_{\text{SL}'+F}}{\text{DR}_{\text{SL}'}} = 1 + \text{SL}' K_{\text{SL}''} (K_{\text{SL}'} = 1.5 \times 10^{10} M^{-1} \text{ and } K_F = 3.6 \times 10^4 M^{-1})
\]

\[
\frac{\text{DR}_{\text{SL}'+F}}{\text{DR}_{\text{SL}''}} / \frac{\text{DR}_{\text{SL}'+F}}{\text{DR}_{\text{SL}'}} = \frac{F}{K_F}
\]

\[
\frac{A_{\text{F}+\text{SL}''}/A_{\text{SL}'}}{A_{\text{F}+\text{SL}''}/A_{\text{SL}''}} \text{ is the observed dose ratio, } A_{\text{F}+\text{SL}''}/A_{\text{SL}'} \text{ being the concentration of agonist required to produce the same response in the presence of F & SL'' as } A_{\text{SL}''} \text{ in the presence of SL'. If the two antagonists are competitive, } A_{\text{F}+\text{SL}''}/A_{\text{SL}'} \text{ should be equal to } \frac{\text{DR}_{\text{SL}'+F}}{\text{DR}_{\text{SL}''}} / \frac{\text{DR}_{\text{SL}'+F}}{\text{DR}_{\text{SL}'}}.
\]

**Details**

Preparation - intact pieces of ileum

Bathing solution - Tyrode's

Lever - isotonic

Agonist - carbachol

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>SL'</th>
<th>SL''</th>
<th>F</th>
<th>t_{off}</th>
<th>t_{on}</th>
<th>(\frac{A_{\text{F}+\text{SL}''}}{A_{\text{SL}''}})</th>
<th>(\frac{\text{DR}<em>{\text{F}+\text{SL}''}}{\text{DR}</em>{\text{F}}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>198 I</td>
<td>10 x 10^{-10}</td>
<td>92 x 10^{-10}</td>
<td>1 x 10^{-4}</td>
<td>no transitional stage</td>
<td>4.7</td>
<td>4.6</td>
<td></td>
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<tr>
<td>198 II</td>
<td>20 x 10^{-10}</td>
<td>308 x 10^{-10}</td>
<td>4 x 10^{-4}</td>
<td>very small transition</td>
<td>23.0</td>
<td>15.4</td>
<td></td>
</tr>
<tr>
<td>199 II</td>
<td>40 x 10^{-10}</td>
<td>617 x 10^{-10}</td>
<td>4 x 10^{-4}</td>
<td>25</td>
<td>4.5</td>
<td>12.8</td>
<td>15.4</td>
</tr>
<tr>
<td>Mean ± S.E.M.</td>
<td>59.0± 12.1</td>
<td>29.1±8.9</td>
<td>39.4±3.2</td>
<td>29.8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[
\frac{A_{\text{F}+\text{SL}''}}{A_{\text{SL}''}} = \frac{\text{DR}_{\text{F}+\text{SL}''}}{\text{DR}_{\text{F}}}
\]
Discussion

If the interaction with the receptors is rate-limiting, and the fast antagonist is so fast that it equilibrates immediately with the receptors not occupied by the slow antagonist, no transitional stage would be observed. However as shown in DIAGRAM I.12 and TABLE I.14 a transitional stage was observed.

The transitional stage does not seem to reflect the rate at which the fast antagonist combines with the available receptors because the rates of onset of the concentrations of pentyl TEA used, (see I.4), are too fast to account for the change observed. In addition the size of the initial displacement increased as F increased whereas if the change were due to the onset of pentyl TEA the duration of the transitional stage would be expected to decrease as F increased because the rate of onset would increase as F increased.

The kinetics of BTrMe therefore appear to be limited by access in some way, the kinetics reflecting the rate at which the concentration in the proximity of the receptors rises to that in the bulk of the bathing medium.

In addition the decrease in BTrMe's occupancy in the first minute, assuming that during this time the receptors were exposed to SL' and F, can be compared with the results of I.2, in which a concentration F was superimposed on SL'.

For instance, considering the superimposition of 8 x 10^{-4}M pentyl TEA in I.2 $t_{off}^{DR_F,SL}/DR_F = 75$ mins (from DIAGRAM I.10) whereas in this section, (TABLE I.14), $t_{off}^{DR_F,SL}/DR_F = 59.0 \pm 12.1$ mins, (mean $\pm S.E.M.$ n = 5)

Similarly considering the superimposition of 16 x 10^{-4}M pentyl TEA in I.2 $t_{off}^{DR_F,SL}/DR_F = 45$ mins, whereas in this section
$t_{\text{off}}^{\text{DRF} \cdot \text{SL} / \text{DRF}} = 25.9 \pm 6.1 \text{ (mean} \pm \text{SE, } n = 7)$

The initial displacement is therefore compatible with the receptors being exposed to SL' and F during the first minute, the subsequent re-establishment of equilibrium reflecting the rate at which the concentration of BTrMe in the proximity of the receptors rises to SL".

There did not appear to be any relationship between $t_{\text{on}}'$ calculated from the rate of re-establishment of BTrMe's occupancy and SL', SL" or F.
I.4 AN EXAMINATION OF THE ASSUMPTIONS USED IN PART I

In the previous three sections certain assumptions were made. These assumptions will now be considered.

1) The relationship between BTrMe's occupancy and the apparent dose ratio

In I.1 it was assumed that between $10 \times 10^{-10}$ and $40 \times 10^{-10}$ M BTrMe, the antagonist occupancy at any time can be calculated from the dose ratio at that time:

As $p = \frac{B K_B}{1 + B K_B}$ and $DR = \frac{1 + B K_B}{1 + B K_B}$

Therefore $p = \frac{DR - 1}{DR}$

This assumes that either complete equilibrium between agonist, antagonist and receptors is established in the interval between the addition of the agonist and when it is washed out, and that receptor occupancy by the agonist is negligible in relation to the available receptor pool. Thus whether this relationship applies depends on the concentration of the antagonist, the 'speed' of the antagonist, the efficacy of the agonist and the agonist contact time.

Therefore if the concentration of an antagonist is increased there comes a stage when this relationship no longer applies and as a result the slope and the maximum of the log dose-response relationship will be less than that in the absence of antagonist. In addition the rate of contraction recorded isotonically sometimes becomes slower, Stephenson (1956), and it is apparent that the contact time is not sufficient for the response to reach an equilibrium.

BTrMe is a particularly slow antagonist and therefore it was thought advisable to determine the concentration of BTrMe above which
these 'non-equilibrium' effects were likely to occur. This was done in two ways:

1. The equilibrium dose ratios produced by increasing concentrations of BTrMe were estimated and the relationship between (DR - 1) and SL, the concentration of BTrMe, was compared with that expected: If

\[ (\text{DR}_\infty - 1) = \text{SL} K_{\text{SL}}, \]

there should be a linear relationship between \( \log(\text{DR}_\infty - 1) \) and \( \log(\text{SL}) \).

To avoid the assumption that the antagonist had caused a parallel shift in the log dose-response relationship the equilibrium dose ratio, \( \text{DR}_\infty \), was taken as the ratio of the concentration of agonist in the presence of the antagonist required to produce the same response as \( 2 \times 10^{-7} \) M carbachol in the absence of antagonist, and \( 2 \times 10^{-7} \) M.

2. In addition an estimation was made of the change in slope of the log-dose response relationship: the gradient of the log dose-response relationship in the absence of antagonist was determined at the response level of \( 2 \times 10^{-7} \) M carbachol. The gradient in equilibrium with the antagonist was also determined at the same response level. The gradient ratio was then the ratio of the gradient in the presence of the antagonist to that in the absence. This ratio would therefore be 1 if there had been a parallel shift in the log dose-response curve and less than 1 if the slope had decreased.

The equilibrium dose ratios produced by various concentrations of BTrMe were estimated using carbachol and the methods of 1.1. In addition the gradient ratio was determined in each experiment.

**TABLE 1.15** shows the results of most of these experiments - it does not include 4 estimations made using \( 100 \times 10^{-10} \) M BTrMe and 2 estimations made using \( 200 \times 10^{-10} \) M BTrMe because in these experiments
**TABLE I.15 & DIAGRAM I.16:** The relationship between the concentration of BTrMe and the equilibrium dose ratio (DR\(_\infty\)) produced

<table>
<thead>
<tr>
<th>Concentration of BTrMe (M)</th>
<th>(n)</th>
<th>Mean (DR(_\infty) - 1) ± S.E.M.</th>
<th>Mean Gradient Ratio ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) 10 \times 10^{-10}</td>
<td>(10)</td>
<td>12.6 ± 1.6</td>
<td>1.15 ± 0.07</td>
</tr>
<tr>
<td>20 \times 10^{-10}</td>
<td>(5)</td>
<td>33.2 ± 3.6</td>
<td>0.90 ± 0.14</td>
</tr>
<tr>
<td>40 \times 10^{-10}</td>
<td>(3)</td>
<td>150 ± 37.6</td>
<td>1.14 ± 0.64</td>
</tr>
<tr>
<td>100 \times 10^{-10}</td>
<td>(7)</td>
<td>341 ± 40.0</td>
<td>1.00 ± 0.18</td>
</tr>
<tr>
<td>200 \times 10^{-10}</td>
<td>(2)</td>
<td>455 ± 45.0</td>
<td>0.93 ± 0.28</td>
</tr>
<tr>
<td>(b) 10 \times 10^{-10}</td>
<td>(6)</td>
<td>16.4 ± 3.4</td>
<td>1.13 ± 0.19</td>
</tr>
<tr>
<td>28 \times 10^{-10}</td>
<td>(2)</td>
<td>38 ± 18.8</td>
<td>1.01 ± 0.11</td>
</tr>
<tr>
<td>39 \times 10^{-10}</td>
<td>(1)</td>
<td>91</td>
<td>0.94</td>
</tr>
</tbody>
</table>

SL is the concentration of BTrMe

(n), number of estimations, each using ileum from a different guinea-pig

The Gradient Ratio = 1 if there was a parallel shift in the log dose response curve, and is < 1 if the slope has decreased in the presence of antagonist. (for definition see text)

**Details**

Preparation - intact pieces of ileum
Bathing solution - Tyrode's
Lever - isotonic
Antagonist - BTrMe

(a) Agonist - carbachol
(b) Agonist - penty1 TMA

N.B. agonist contact time 17 secs.
when the tissue was in equilibrium with the antagonist, the contractions were obviously not reaching an equilibrium in the 17 seconds contact time. For the same reason 5 estimations made using BETMe concentrations between $300 \times 10^{-10}M$ and $600 \times 10^{-10}M$ are not included. (The mean gradient ratio of these experiments was $0.71 \pm 0.6$ (mean±S.E.M.) )

The relationship between $\log(DR_{\infty} - 1)$ and $\log(SL)$ is shown in DIAGRAM I.16 for the results given in TABLE I.15.

A consideration of the relationship between $\log(DR_{\infty} - 1)$ and $\log(SL)$, and also of the gradient ratios shown in TABLE I.14 and the results not included in TABLE I.15, indicate that non-equilibrium effects were unlikely to have interfered with the kinetic experiments of I.1 in which the agonist carbachol was used together with concentrations of BETMe between $10 \times 10^{-10}M$ and $40 \times 10^{-10}M$. It therefore seems reasonable to estimate antagonist occupancy from the dose ratio as described previously, in these experiments.

However if greater concentrations of BETMe are used, non-equilibrium effects become increasingly likely.

In I.3 concentrations of BETMe considerably larger than $40 \times 10^{-10}M$ were used, but because these concentrations of BETMe were used in combination with another antagonist, BETMe's occupancy was no greater than that produced by $40 \times 10^{-10}M$ BETMe acting alone. Therefore non-equilibrium effects would not necessarily occur in these experiments even with such large concentrations of BETMe, Ginsborg & Stephenson (1974), and indeed non-equilibrium effects were not observed.
However the possibility was considered that the slow responses and the change in slope of the log dose-response relationship were not due to the slowness of the antagonist but to some other concentration dependent effect. Therefore the change in slope of the log dose-response relationship and the slowness of the responses in the presence of high concentrations of BTrMe were examined to see if they were consistent with their being due to non-equilibrium effects:

1. Non-equilibrium effects would be expected to occur at lower concentrations of BTrMe if an agonist of lower efficacy than carbachol was used. Therefore the equilibrium dose ratios produced by various concentrations of BTrMe were estimated as before but using the agonist pentyltrimethyl ammonium (pentyl TMA), this agonist having a lower efficacy than carbachol. In this case the dose ratio was the ratio of the concentration of agonist in the presence of antagonist required to produce the same response as $2 \times 10^{-6} \text{M}$ pentyl TMA in the absence of antagonist, to $2 \times 10^{-6} \text{ M}$. The gradient ratio was also determined as before but at the response level of $2 \times 10^{-6} \text{M}$ pentyl TMA.

TABLE I.15 shows these values. However it does not include 12 estimations made using BTrMe concentrations between $50 \times 10^{-10} \text{M}$ and $200 \times 10^{-10} \text{M}$ because the mean gradient ratio of these estimations was $0.46 \pm 0.15$ (mean $\pm$ S.E.M.), and because the contractions were obviously not reaching an equilibrium in the contact time.

Non-equilibrium effects were therefore found to interfere when the concentration of BTrMe was $50 \times 10^{-10} \text{M}$ and above when the agonist was pentyl TMA. This compares with $100 \times 10^{-10} \text{M}$ and above when carbachol was used. This difference is therefore consistent with the decrease in slope in the presence of high concentrations of BTrMe being due to non-equilibrium effects.
2. In addition if the change in slope and maximum of the carbachol log dose-response curve when high concentrations of BTrMe were used, were due to non-equilibrium effects, the log dose-response curve of pentyl TMA would be expected to be depressed more than that of carbachol and that of hexyl TMA more than that of pentyl TMA, hexyl TMA having a lower efficacy than pentyl TMA.

Therefore dose response curves were obtained in the presence and absence of various concentrations of BTrMe, using the agonists carbachol, pentyl TMA and also hexyl TMA. Dose response curves were obtained by pipetting a small volume < 0.2 ml of the agonist solution into the organ bath, washout occurring automatically after 17 seconds. The concentration was then doubled progressively and the actual concentration was estimated by comparing the responses produced by injection with those produced when the organ bath was overflowed with a solution of known concentration.

DIAGRAM I shows the effect of 50 x \(10^{-10}\) M BTrMe on the log dose-response relationships of carbachol, pentyl TMA and hexyl TMA as found in experiment 64. As expected the slope and the maximum of the hexyl TMA curve was depressed more than that of the pentyl TMA curve, and the pentyl TMA curve more than that of carbachol. Similar effects were also found in other experiments.

3. Also if the slowness of the response to carbachol, in the presence of high concentrations of BTrMe was due to non-equilibrium effects, the responses to pentyl TMA would be expected to take even longer to reach an equilibrium.

The effect of increasing the agonist contact time was therefore investigated in a few experiments. For instance in experiment 46 II, it was observed that in the presence of 100 x \(10^{-10}\) M BTrMe the responses to pentyl TMA did not reach an equilibrium in the 17 seconds.
Diagram 1.17: The effect of $50 \times 10^{-10} \text{M BTrMe}$ on the log dose-response curves obtained with the agonists carbachol, pentyl TMA and hexyl TMA.

Details
Experiment 64 I
Preparation - intact pieces of ileum
Bathing solution - Tyrode's
Lever - isotonic
Antagonist - $50 \times 10^{-10} \text{M BTrMe}$
Agonists - Carbachol $\times$
Pentyl TMA $\circ$
Hexyl TMA $\ast$
contact time, and with this contact time the apparent gradient ratio was 0.39. However if the contact time was increased to 45 seconds, an equilibrium was apparently reached, as shown in Diagram I.18, and the gradient ratio was then 0.95.

These experiments therefore support the view that the change in slope of the log dose-response relationship and the slow contractions in the presence of large concentrations of BTrMe are due to non-equilibrium effects.

\[ \text{ii) The equilibrium dose ratio produced by various concentrations of BTrMe} \]

In I.2 and I.3 the equilibrium dose ratios produced by certain concentrations of BTrMe were estimated using the relationship:

\[ DR_{SL} = \frac{1 + SL}{K_{SL}} \]

and taking

\[ K_{SL} = 1.5 \times 10^{-10} M^{-1} \]

The justification for using the first relationship has already been discussed and so the value of \( K_{SL} \) will now be considered.

\( K_{SL} \) was calculated from the 27 estimations of \( DR_{\infty} \) using carbachol shown in Table I.15 and was found to be \( 1.55 \pm 0.17 \times 10^{-10} M^{-1} \). This value was therefore used for the calculations in I.2 and I.3.

However affinity constant estimations made of a slow antagonist such as BTrMe are bound to have a greater uncertainty than those of faster antagonists, because of the longer time lapse between the addition of the antagonist and the establishment of equilibrium. This time can be reduced by using higher concentrations of the slow antagonist, but when higher concentrations of a slow antagonist are used there is also an increased likelihood of non-equilibrium effects occurring.
DIAGRAM I.18: Comparing the rate of response to 320 x 10^{-6}M pentyl TMA and 80 x 10^{-7}M carbachol in the presence of 100 x 10^{-10}M BTrMe

Details
Experiment 46 II
Preparation - intact pieces of ileum
Bathing solution - Tyrode's
Lever - isotonic

* The paper drive was automatically switched off after washout and restarted just before the agonist was added in the following cycle.
The concentrations of BTrMe in TABLE I,15 were between $10 \times 10^{-10} \text{M}$ and $200 \times 10^{-10} \text{M}$ and even at the highest of these concentrations about an hour was required for the establishment of equilibrium and higher concentrations of BTrMe could not be used for affinity constant estimations due to the likelihood of non-equilibrium effects.

However in the experiments of I.3 the time lapse is much shorter. The observed dose ratios of these experiments were also found to be consistent with $K_{SL}^{-1}$ being equal to $1.5 \times 10^{10} \text{M}^{-1}$ as shown in TABLE I,14: the observed dose ratios $A_{SL+F}/A_{SL}$ agree with those calculated assuming that $A_{SL+F} = 1 + SL K_{SL}^{-1} F K_F$, $K_{SL} = 1.5 \times 10^{10} \text{M}^{-1}$

and $K_F = 3.6 \times 10^4 \text{M}^{-1}$.

The value $1.5 \times 10^{10} \text{M}^{-1}$ was therefore used as the affinity constant of BTrMe despite the fact that this antagonist was also investigated by Barlow and Mustafa (1968) and they obtained a value of $2.36 \times 10^{10} \text{M}^{-1}$. The difference between this value and that obtained in this study may be just variation between samples or may be due to the very much higher concentrations of BTrMe used by Barlow & Mustafa and associated with this, the shorter time lapse involved when higher concentrations are used. This last possibility seems unlikely because the observed dose ratios in I.3 are consistent with $K_{SL}^{-1}$ being $1.5 \times 10^{10} \text{M}^{-1}$ and there were much shorter time lapses in these experiments. It is also notable that Barlow & Mustafa (1958) do not mention observing non-equilibrium effects even when they used concentrations of BTrMe above $1000 \times 10^{-10} \text{M}$. 
iii) The dose ratio produced by pentyl TEA and BTrMe together

In 1.2, \( DR_{P+SL} \) was calculated from the relationship:

\[
DR_{P+SL} = 1 + SL \frac{K_{SL}}{F} K_F^P
\]

taking \( K_{SL} \) to be \( 1.5 \times 10^{10} \text{M}^{-1} \) and \( K_F \) to be \( 3.6 \times 10^{4} \text{M}^{-1} \). The justification for using this value of \( K_{SL} \) has already been discussed. The value of \( K_F \) will now be considered:

The affinity constant of pentyl TEA was estimated from the equilibrium dose ratio produced by various concentrations of pentyl TEA, using the methods described in 1.1. TABLE 1.19 summarizes the results of these experiments, and the relationship between \( (\text{DR}_\infty - 1) \) and the concentration of pentyl TEA is shown in DIAGRAM 1.20.

When the concentration of pentyl TEA is below \( 8 \times 10^{-4} \text{M} \) there appears to be a reasonably linear relationship between \( (\text{DR}_\infty - 1) \) and the concentration, \( F \), as expected from the relationship:

\[
(\text{DR}_\infty - 1) = F K_F
\]

and the mean value of \( K_F \) calculated from these 24 estimations was \( 3.69 \pm 0.15 \times 10^{4} \text{M}^{-1} \) (mean \( \pm \) S.E.M.).

However when the concentration of pentyl TEA was greater than \( 8 \times 10^{-4} \text{M} \), the observed equilibrium dose ratios were lower than expected from the above relationship.

This phenomenon is puzzling. It was not found to be associated with a change in the slope of the log dose-response curves of carbachol, pentyl TMA or hexyl TMA. In addition the combined dose ratio produced by BTrMe and high concentrations of pentyl TEA appear to be consistent with \( K_F \) being equal to \( 3.6 \times 10^{4} \text{M}^{-1} \). This is shown in TABLE 1.14 for the experiments of 1.3 and also in TABLE 1.21 for those experiments in 1.2 in which the interaction between BTrMe and pentyl TEA was followed for long enough for an
The relationship between the concentration of pentyl TEA and the equilibrium dose ratio produced, $DR_{\infty}$

<table>
<thead>
<tr>
<th>Antagonist Concentration M</th>
<th>(n)</th>
<th>Mean ($DR_{\infty}$) ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$0.3 \times 10^{-4}$</td>
<td>(3)</td>
<td>$2.8 \pm 0.5$</td>
</tr>
<tr>
<td>$1 \times 10^{-4}$</td>
<td>(3)</td>
<td>$4.4 \pm 0.2$</td>
</tr>
<tr>
<td>$2.5 \times 10^{-4}$</td>
<td>(7)</td>
<td>$10.9 \pm 0.4$</td>
</tr>
<tr>
<td>$4 \times 10^{-4}$</td>
<td>(4)</td>
<td>$14.2 \pm 0.4$</td>
</tr>
<tr>
<td>$5 \times 10^{-4}$</td>
<td>(3)</td>
<td>$19.1 \pm 2.2$</td>
</tr>
<tr>
<td>$8 \times 10^{-4}$</td>
<td>(4)</td>
<td>$28.5 \pm 3.5$</td>
</tr>
<tr>
<td>$15 \times 10^{-4}$</td>
<td>(4)</td>
<td>$44.1 \pm 4.7$</td>
</tr>
<tr>
<td>$20 \times 10^{-4}$</td>
<td>(2)</td>
<td>$44.4 \pm 0.6$</td>
</tr>
<tr>
<td>$25 \times 10^{-4}$</td>
<td>(3)</td>
<td>$52.2 \pm 5.7$</td>
</tr>
<tr>
<td>$30 \times 10^{-4}$</td>
<td>(6)</td>
<td>$53.7 \pm 4.5$</td>
</tr>
</tbody>
</table>

(n) number of estimations made, each using ileum from a different guinea-pig

Details
Preparation - intact pieces of ileum
Bathing Solution - Tyrode's
Lever - isotonic
Antagonist - pentyl TEA
Agonist - carbachol
TABLE I.21: Comparing the observed equilibrium dose ratio with that calculated, for those experiments in 1.2 where the interaction between pentyl TEA & BTrMe was followed to an equilibrium.

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Conc. of BTrMe SL</th>
<th>Conc. of Pentyl TEA F</th>
<th>Observed Dose Ratio</th>
<th>Calculated Dose Ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>( \frac{A_{SL} + F}{A_{SL}} )</td>
<td>( \frac{1 + SL K_{SL}^* F K_F}{1 + SL K_{SL}} )</td>
</tr>
<tr>
<td>112 I</td>
<td>20 x 10^{-10}</td>
<td>2.5 x 10^{-4}</td>
<td>1.2</td>
<td>1.3</td>
</tr>
<tr>
<td>127 X</td>
<td>20 x 10^{-10}</td>
<td>2.5 x 10^{-4}</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>199 X</td>
<td>20 x 10^{-10}</td>
<td>2.5 x 10^{-4}</td>
<td>1.7</td>
<td>1.3</td>
</tr>
<tr>
<td>113 I</td>
<td>60 x 10^{-10}</td>
<td>2.5 x 10^{-4}</td>
<td>1.2</td>
<td>1.1</td>
</tr>
<tr>
<td>114 X</td>
<td>60 x 10^{-10}</td>
<td>2.5 x 10^{-4}</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>142 II</td>
<td>40 x 10^{-10}</td>
<td>4.8 x 10^{-4}</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>146 II</td>
<td>40 x 10^{-10}</td>
<td>4.8 x 10^{-4}</td>
<td>1.4</td>
<td>1.3</td>
</tr>
<tr>
<td>159 II</td>
<td>40 x 10^{-10}</td>
<td>4.8 x 10^{-4}</td>
<td>1.4</td>
<td>1.3</td>
</tr>
<tr>
<td>160 II</td>
<td>40 x 10^{-10}</td>
<td>4.8 x 10^{-4}</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>115 I</td>
<td>10 x 10^{-10}</td>
<td>14 x 10^{-4}</td>
<td>4.6</td>
<td>3.7</td>
</tr>
<tr>
<td>116 X</td>
<td>6 x 10^{-10}</td>
<td>25 x 10^{-4}</td>
<td>18.7</td>
<td>6.1</td>
</tr>
<tr>
<td>162 X</td>
<td>6 x 10^{-10}</td>
<td>25 x 10^{-4}</td>
<td>18.7</td>
<td>6.1</td>
</tr>
<tr>
<td>113 X</td>
<td>10 x 10^{-10}</td>
<td>25 x 10^{-4}</td>
<td>9.9</td>
<td>4.2</td>
</tr>
<tr>
<td>115 X</td>
<td>10 x 10^{-10}</td>
<td>25 x 10^{-4}</td>
<td>8.2</td>
<td>4.2</td>
</tr>
<tr>
<td>166 I</td>
<td>40 x 10^{-10}</td>
<td>35 x 10^{-4}</td>
<td>3.9</td>
<td>3.1</td>
</tr>
<tr>
<td>166 X</td>
<td>40 x 10^{-10}</td>
<td>35 x 10^{-4}</td>
<td>4.1</td>
<td>3.1</td>
</tr>
<tr>
<td>163 X</td>
<td>40 x 10^{-10}</td>
<td>35 x 10^{-4}</td>
<td>3.7</td>
<td>3.1</td>
</tr>
<tr>
<td>164 I</td>
<td>40 x 10^{-10}</td>
<td>45 x 10^{-4}</td>
<td>3.8</td>
<td>3.7</td>
</tr>
<tr>
<td>164 X</td>
<td>40 x 10^{-10}</td>
<td>45 x 10^{-4}</td>
<td>3.9</td>
<td>3.7</td>
</tr>
</tbody>
</table>

The observed dose ratio is the ratio of the concentration of agonist in equilibrium in the presence of SL and F, \( A_{SL} + F \), required to produce the same response as \( A_{SL} \) in the presence of SL only.

If the antagonists compete with one another, the ratio \( \frac{A_{SL} + F}{A_{SL}} \) should equal \( \frac{(1 + SL K_{SL}^* F K_F)}{(1 + SL K_{SL})} \). This ratio is shown above assuming \( K_{SL} = 1.5 \times 10^{11} M^{-1} \) and \( K_F = 3.6 \times 10^{-4} M^{-1} \).

The ratio \( \frac{(1 + SL K_{SL}^* F K_F)}{(1 + SL K_{SL})} \) should also be equal to \( \frac{(DR_F + SL K_{SL})}{(1 + SL K_{SL})} \) where \( DR_F \) is the dose ratio produced by a concentration \( F \) of the fast antagonist acting alone. This ratio is also shown above assuming \( K_{SL} = 1.5 \times 10^{11} M^{-1} \) and using the values of \( DR_F \) determined experimentally, (from DIAGRAM I.20)

Details
(see also I.2)
Preparation - intact pieces of ileum
Bathing solution - Tyrode's
Lever - isotonic
Antagonists - BTrMe & pentyl TEA
Agonist - carbachol
equilibrium to be established.

As the experimental dose ratios do seem to correspond more nearly with that calculated assuming $K_P = 3.6 \times 10^4 M^{-1}$ than that calculated using the experimentally determined value of DR for the particular concentration of the fast antagonist, this value of $K_P$ was used to calculate DR$_{P+SL}$.

In I.3 it was also assumed that if $SL'' = SL' (1 + F K_P)$, $K_P$ being $3.6 \times 10^4 M^{-1}$, then $p_{SL'} = p_{SL''}$. The reasonable agreement between the equilibrium dose ratio found experimentally and that calculated assuming $K_P = 3.6 \times 10^4 M^{-1}$, as shown in TABLE I.14, is consistent with this assumption.

iv) The speed of the fast antagonist pentyl TEA

In I.2 it was assumed that the difference in rates between the slow and the fast antagonist was sufficiently great that the fast antagonist could be considered to be in equilibrium at all times with the receptors not occupied by the slow antagonist. Therefore:

$$P_F = \frac{F K_P (1 - p_{SL})}{1 + F K_P}$$

In order to determine whether this assumption was justified the kinetics of onset and offset of various concentrations of pentyl TEA were examined, using the methods described in I.1.

The rate of onset was so fast that even with the lowest concentration, $0.3 \times 10^{-4} M$, the first response in the presence of the antagonist, corresponding to an antagonist exposure time of 17 seconds, was not sufficiently larger than the subsequent responses for $t_{on}$ to be estimated. It was for this reason that the transitional
stage observed in I.3 can not reflect the onset of the fast antagonist.

The rate of offset was however slow enough, even when concentrations as low as $0.3 \times 10^{-4} \text{M}$ were used, for the rate of decrease of occupancy to be examined. The results of these experiments are shown in Diagram I.23 and Table I.22. Contrary to the predictions of the interaction limited model there does not appear to be a linear relationship between log occupancy and time, the rate of offset becoming slower with time. In addition the initial rate of offset was calculated from the first response in the presence of the antagonist. The time constants corresponding to this rate are given in Table I.20 and it appears that the initial rate of offset is slower following the antagonism of higher concentrations of pentyl TEA. This is not associated with different exposure times to the antagonist; the tissue was usually exposed to the antagonist for 7 cycles, i.e., 630 seconds. Also, increasing the exposure time to $0.3 \times 10^{-4} \text{M}$ did not decrease the subsequent rate of offset. In addition the difference in rates can not be explained by the change in the apparent affinity of pentyl TEA as its concentration increases; a decrease in the apparent affinity due to an increase in the value of $k_2$ would cause an increased rate of offset, if dissociation was rate-limiting and this is the opposite to the change observed. Also the rate of offset gets appreciably slower when the concentration of pentyl TEA is increased from $0.3 \times 10^{-4}$ to $8 \times 10^{-4} \text{M}$, although little change in the apparent affinity constant was observed below $8 \times 10^{-4} \text{M}$.

However the pentyl TEA-receptor dissociation rate constant must be larger than $1/16.83 \text{ s}^{-1}$, 16.83 being the lowest value of
TABLE I. 22 & DIAGRAM I. 23; The rate of offset of pentyl TEA

<table>
<thead>
<tr>
<th>Concentration of pentyl TEA (M)</th>
<th>n</th>
<th>Mean DR_{CO} \pm S.E.M.</th>
<th>Mean 'initial' t_{OFF} \pm S.E.M. (secs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(0.3 \times 10^{-4})</td>
<td>(4)</td>
<td>2.8 \pm 0.5</td>
<td>16.8 \pm 4.1</td>
</tr>
<tr>
<td>(1.0 \times 10^{-4})</td>
<td>(3)</td>
<td>4.4 \pm 0.2</td>
<td>18.1 \pm 1.9</td>
</tr>
<tr>
<td>(2.5 \times 10^{-4})</td>
<td>(4)</td>
<td>11.1 \pm 0.6</td>
<td>33.2 \pm 8.8</td>
</tr>
<tr>
<td>(4.0 \times 10^{-4})</td>
<td>(4)</td>
<td>14.2 \pm 0.4</td>
<td>44.4 \pm 12.3</td>
</tr>
<tr>
<td>(8.0 \times 10^{-4})</td>
<td>(5)</td>
<td>27.9 \pm 2.8</td>
<td>36.3 \pm 8.2</td>
</tr>
<tr>
<td>(20.0 \times 10^{-4})</td>
<td>(2)</td>
<td>44.4 \pm 0.6</td>
<td>35.1</td>
</tr>
<tr>
<td>(30.0 \times 10^{-4})</td>
<td>(4)</td>
<td>49.4 \pm 5.2</td>
<td>59.4 \pm 17.4</td>
</tr>
</tbody>
</table>

Mean values of (p_F) are plotted on a log scale against time

Details (see also text)
- Preparation - intact pieces of ileum
- Bathing solution - Tyrode's
- Lever - isotonic
- Antagonist - pentyl TEA, concentrations \((M) \times 10^4\) are indicated - ( )
- Agonist - carbachol
observed. In 1.2 it was found that the BTrMe-receptor
dissociation rate constant must be larger or equal to $1/(20 \times 60)$ s$^{-1}$. The ratio of these values is 270, and so if the kinetics of these
antagonists were limited by their rate of interaction with the
receptors, the size of this ratio would justify the assumption
that the fast antagonist is at all times in equilibrium with the
receptors not occupied by the slow antagonist.

However neither the kinetics of pentyl TEA nor BTrMe are
compatible with an interaction-limited situation. Also the lack of
any particular value for the dissociation constants of BTrMe and
pentyl TEA made it impossible to predict what would happen if
interaction were rate-limiting and if the difference in rates
was not sufficiently large for the assumption to be reasonably made.
The kinetics of BTrMe, under the conditions used, do not appear to be limited by the rate at which it interacts with the receptors, the following observations being incompatible with the predictions of the interaction-limited model:

1. Occupancy does not appear to increase exponentially with time during the onset of antagonism.

2. The time constant for the decrease in occupancy with time, when the antagonist is removed, is not independent of the concentration of the antagonist.

3. The time constant for the decrease in occupancy of BTrMe when a concentration of the fast antagonist is superimposed, multiplied by the ratio \( \frac{DR_{P+SL}}{DR_F} \) is not independent of the concentration of the fast antagonist.

4. A transitional stage was observed when the interaction between BTrMe and pentyl TEA was examined in conditions where the concentration of BTrMe was adjusted so that its occupancy in the absence of pentyl TEA was the same as that in equilibrium in the presence of the pentyl TEA.

In addition the variation in \( t_{off}^{DR_{P+SL}/DR_F} \) from one experiment to another, and the variation in the concentration of BTrMe producing non-equilibrium effects, are difficult to explain if interaction were rate limiting. On the other hand such variation might be expected if access was rate limiting.

The one experimental situation where the kinetics of BTrMe could be interaction limited is the decrease in BTrMe's occupancy following the superimposition of very large concentrations of
pentyl TEA. This is considered further in part III.

In addition the kinetics of offset of the very fast antagonist pentyl TEA are also not compatible with the predictions of an interaction limited situation, the time constant for the rate of offset getting larger as the concentration of pentyl TEA is increased. This contrasts with the time constant of the rate of offset of BTrMe which got smaller as the concentration of BTrMe was increased.

Thus having shown that the kinetics of BTrMe are access limited, the next step was to establish that faster antagonists were also access-limited. Although the experiments with the very fast antagonist pentyl TEA showed that this compound was also access limited, it is too fast for the rate of onset to be followed. Therefore the antagonist lachesine was investigated, this compound being intermediate in speed between BTrMe and pentyl TEA. In addition lachesine was one of the compounds examined by Paton & Rang (1965) and so comparisons could be made between their study and this one.
PART II
THE KINETICS OF LACHESINE - are the kinetics of this antagonist access limited?

In order to see whether the kinetic behaviour of lachesine on guinea-pig ileum is similar to that of BTxMe two types of experiments were performed corresponding to those of I.1 and I.2:

1. Firstly the rates of onset and offset of various concentrations of lachesine were followed.

2. Then the decrease in lachesine's occupancy following the superimposition of a concentration of pentyl TEA was examined.

As before, carbachol contractions of intact pieces of ileum in Tyrode's solution were recorded isotonically, (details p.56).

II.1. THE KINETICS OF ONSET AND OFFSET OF LACHESINE

Procedure and Results

The kinetics of three concentrations of lachesine were examined, \((0.5 \times 10^{-6} \text{M}, 1 \times 10^{-6} \text{M} \text{ and } 2 \times 10^{-8} \text{M})\), using the procedure illustrated in DIAGRAM I.1.

Several experiments were done using each concentration of lachesine and the mean occupancy at each response time was calculated from the results of the individual experiments. These mean values are shown in DIAGRAM II.1, plotted as described on p.60. Contrary to the predictions of the interaction limited model there does not appear to be a linear relationship between occupancy plotted on a log scale and time. This is much more marked than the deviation observed in equivalent experiments in I.1 when the antagonist was BTxMe.
DIAGRAM II.1: The kinetics of onset and offset of lachesine

For onset mean values of $(p_\text{oo} - p_\text{c}) \pm \text{S.E.M.}$ are plotted on a log scale against time.

For offset mean values of $(p_\text{c}) \pm \text{S.E.M.}$ are plotted on a log scale against time.

Details
(see also TABLE II.2)
Preparation - intact pieces of ileum
Bathing solution - Tyrode's
Lever - isotonic

Antagonist - lachesine $0.5 \times 10^{-8} \text{M}$ — $2 \times 10^{-8} \text{M}$

$n=5$ $DR_\text{oo}=4.9\pm0.4$ (mean $\pm$ S.E.M)
$n=7$ $DR_\text{oo}=7.4\pm0.8$
$n=16$ $DR_\text{oo}=14.9\pm1.0$

Agonist - carbachol
Time constants were calculated for each experiment from the first three responses after the antagonist was added or removed, i.e. the mean rate of the first three responses was used as an estimate of the initial rate. These values are shown in TABLE II.2. Although $t_{\text{off}}/t_{\text{on}}$ is approximately equal to $DR_{\infty}$ when the concentration of lachesine is $0.5 \times 10^{-8} \text{M}$, the difference between $t_{\text{off}}/t_{\text{on}}$ and $DR_{\infty}$ increases as the concentration of lachesine is increased. This is contrary to the predictions of the interaction limited model. However, in contrast with the results of BTRMe in I.1, $t_{\text{off}}$ appears to be independent of the concentration of lachesine used.

II.2 THE RATE OF OFFSET OF LACHESINE ON SUPERIMPOSITION OF PENTYL TEA

Procedure and Results

The interaction between lachesine and pentyl TEA was investigated as in I.2, except that $DR_{\text{SL}}$, the equilibrium dose ratio produced by lachesine was determined in each experiment. In addition, the interaction between lachesine and pentyl TEA was followed to an equilibrium thus enabling $DR_{\text{P+SL}}$ to be determined rather than calculated as was necessary in I.2.

Three concentrations of lachesine were examined, $0.5 \times 10^{-8} \text{M}$, $1 \times 10^{-8} \text{M}$ and $2 \times 10^{-8} \text{M}$, and also three concentrations of pentyl TEA, $2.5 \times 10^{-4} \text{M}$, $15 \times 10^{-4} \text{M}$ and $25 \times 10^{-4} \text{M}$.

For each combination of lachesine and pentyl TEA, lachesine's mean occupancy corresponding to each response after the fast antagonist was added was calculated from the results of the individual experiments. These mean values and their standard errors are shown in DIAGRAMS II.3-5. A dashed line is superimposed on these diagrams to correspond to an apparent value of $1/k_2$ of 4 minutes. (The limiting value indicated in
**TABLE II.2: The initial rates of onset and offset of lachesine**

| Exp. No. | Antagonist Conc. C/M | Time constant for the initial rate of onset $t_{on}$ mins | Time constant for the initial rate of offset $t_{off}$ mins | $t_{off}/t_{on}$ | Equilibrium Dose ratio DR
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>205 I</td>
<td>$0.5 \times 10^{-8}$</td>
<td>5.3</td>
<td>16.9</td>
<td>3.2</td>
<td>4.6</td>
</tr>
<tr>
<td>206 I</td>
<td>4.9</td>
<td>31.3</td>
<td>6.4</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>209 IX</td>
<td>6.4</td>
<td>28.1</td>
<td>4.4</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>212 IX</td>
<td>7.8</td>
<td>29.5</td>
<td>3.8</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>213 IX</td>
<td>9.5</td>
<td>21.9</td>
<td>2.3</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>Mean ± SE of Mean:</td>
<td>6.7 ± 0.9</td>
<td>25.3 ± 2.7</td>
<td>4.8 ± 0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>204 I</td>
<td>$2 \times 10^{-8}$</td>
<td>4.9</td>
<td>26.6</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>206 I</td>
<td>4.7</td>
<td>19.9</td>
<td>3.0</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>209 IX</td>
<td>6.2</td>
<td>10.7</td>
<td>1.9</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>211 IX</td>
<td>4.9</td>
<td>13.0</td>
<td>2.1</td>
<td>6.8</td>
<td></td>
</tr>
<tr>
<td>212 IX</td>
<td>7.3</td>
<td>43.1</td>
<td>5.7</td>
<td>9.5</td>
<td></td>
</tr>
<tr>
<td>Mean ± SE of Mean:</td>
<td>5.7 ± 0.4</td>
<td>22.3 ± 4.4</td>
<td>7.4 ± 0.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$t_{off}$ and $t_{on}$ were calculated from the first three responses after the addition or removal of the antagonist, assuming an exponential relationship between occupancy and time.

**Details**

**Preparation** - intact pieces of ileum
**Bathing Solution** - Tyrode's
**Lever** - isotonic
**Antagonist** - lachesin
**Agonist** - carbachol

This table includes results, indicated *, which were obtained as described in **III.4**.
**Diagram II.3:** The rate of offset of lachesine when $2.5 \times 10^{-4}M$ pentyl TEA superimposed

Mean values of $(p_t - p_{oo}) \pm S.E.M.$ are plotted on a log scale against time.

**Details**
(see also TABLE II.6)
Preparation - intact pieces of ileum
Bathing solution - Tyrode's
Lever - isotonic
Antagonist - lachesine
$0.5 \times 10^{-8}M$, $n=3$
$1 \times 10^{-8}M$, $n=3$
$2 \times 10^{-8}M$, $n=3$

Pentyl TEA $2.5 \times 10^{-4}M$

Agonist - carbachol

$\ldots$ corresponds to a time constant $t_{off} = 4 \times [\frac{mean \ DR_{p+SL} - mean \ DR_{p+SL+1}}{mean \ DR_{p+SL}}] \text{ mins}$
**Diagram II.6:** The rate of offset of lachesine when $15 \times 10^{-4}$M pentyl TEA superimposed

Mean values of $(p_t - p_\infty)$ ± S.E.M. are plotted on a log scale against time

--- corresponds to a time constant $t_{off} = 4 \times \frac{[\text{mean } DR_{PTEA} - \text{mean } DR_{SL}]}{[\text{mean } DR_{PTEA}]}$ mins

Details
(see also TABLE II.6)
Preparation - intact pieces of ileum
Bathing solution - Tyrode's
Lever - isotonic

Antagonist - lachesine $0.5 \times 10^{-8}$M
$1 \times 10^{-8}$M
$2 \times 10^{-8}$M

Agonist - carbachol $15 \times 10^{-6}$M
Diagram II.5: The rate of offset of lachesine when $25 \times 10^{-4} \text{M}$ pentyl TEA superimposed.

Mean values of $(p_t - p_{oo}) \pm \text{S.E.M.}$ are plotted on a log scale against time.

--- corresponds to a time constant $t_{off} = 4 \times \frac{\text{mean DR_{FLG}} - \text{mean DR}_{SL}}{\text{mean DR}_{FLG}} + 1 \text{ mins}$

Details:
(see also Table II.6)
Preparation - intact pieces of ileum
Antagonist - lachesine $0.5 \times 10^{-5}, 0.1 \times 10^{-5}$
Bathing solution - Tyrode's
Lever - isotonic
Agonist - carbachol

Pentyl TEA $25 \times 10^{-4} \text{M}$
As there does not appear to be a linear relationship between occupancy plotted on a log scale, and time, time constants were calculated from each experiment from the first three responses after the fast antagonist was superimposed, i.e. the mean rate of the first three responses was used as an estimate of the initial rate. Table II.6 shows these values together with the value of 

\[ t_{\text{off}}^{DR_{\text{F}}+\text{SL}}/(DR_{\text{F}}+\text{SL} - DR_{\text{SL}} - 1) \]

As shown on P. 71, if interaction is rate limiting, \( t_{\text{off}}^{DR_{\text{F}}+\text{SL}}/DR_{\text{F}} \) should be equal to \( 1/k_2 \). In these experiments values of \( DR_{\text{F}}+\text{SL} \) and \( DR_{\text{SL}} \) were determined in each experiment. If the two antagonists are competitive 

\[ DR_{\text{F}}+\text{SL} = DR_{\text{F}} + DR_{\text{SL}} - 1 \]

Therefore 

\[ DR_{\text{F}} = DR_{\text{F}}+\text{SL} - DR_{\text{SL}} - 1 \]

Thus the value of 

\[ t_{\text{off}}^{DR_{\text{F}}+\text{SL}}/(DR_{\text{F}}+\text{SL} - DR_{\text{SL}} - 1) \]

should be equal to \( 1/k_2 \) and so be independent of the concentration of pentyl TEA.

However as shown in Diagram II.7 this does not appear to be the case, the value decreasing to what appears to be a limiting value around 4 minutes. This is similar to the behaviour of BTrMe in equivalent experiments in I.2.

The assumptions made in Part II

The assumptions involved in the calculations of this section are similar to those of I:

Lachesine's occupancy, \( p \), was calculated as in I.1 assuming that \( p \) is related to the dose ratio thus: 

\[ p = (DR - 1)/(DR) \]

Since whether non-equilibrium effects occur or not depends on the speed of the antagonist and the efficacy of the agonist such effects would not be expected for a compound as fast as lachesine over the range of concentrations used. As expected, no such effects were observed.

In addition the apparent affinity constants calculated from the
### Table II.6: The rate of decrease of lachesine's occupancy on superimposing pentyl TEA

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Conc. of Lachesine SL M</th>
<th>Conc. of Pentyl TEA F M</th>
<th>$t_{off}$ mins</th>
<th>$\frac{t_{off}}{T_{off}}$</th>
<th>$\frac{DR_{F+SL} - DR_{F}}{T_{off}}$ mins</th>
<th>$\frac{DR_{SL}}{T_{off}}$</th>
<th>$\frac{DR_{F+SL}}{T_{off}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>206 I</td>
<td>0.5 x 10^{-8}</td>
<td>2.5 x 10^{-4}</td>
<td>9.3</td>
<td>13.4</td>
<td>5.3</td>
<td>14.0</td>
<td>14.1</td>
</tr>
<tr>
<td>213 I</td>
<td>1 x 10^{-8}</td>
<td>2.5 x 10^{-4}</td>
<td>4.3</td>
<td>7.0</td>
<td>6.4</td>
<td>14.1</td>
<td>14.1</td>
</tr>
<tr>
<td>215 I</td>
<td>3.3</td>
<td>4.2</td>
<td>4.2</td>
<td>4.7</td>
<td>9.7</td>
<td>17.8</td>
<td>17.8</td>
</tr>
<tr>
<td>Mean ± S.E.M.</td>
<td>5.6 ± 1.9</td>
<td>8.2 ± 2.7</td>
<td>5.5 ± 0.5</td>
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<td></td>
<td></td>
<td></td>
</tr>
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<td>206 II</td>
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<td>9.5</td>
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<td>21.8</td>
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<tr>
<td>Mean ± S.E.M.</td>
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<tr>
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<td>2.5 x 10^{-4}</td>
<td>4.3</td>
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<td>27.0</td>
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<td>21.3</td>
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<tr>
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<td>4.0</td>
<td>9.3</td>
<td>57.0</td>
<td>57.0</td>
<td>57.0</td>
<td>57.0</td>
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<tr>
<td>Mean ± S.E.M.</td>
<td>3.9 ± 0.3</td>
<td>4.5 ± 0.3</td>
<td>6.7 ± 1.4</td>
<td>43.6 ± 6.8</td>
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</tr>
<tr>
<td>210 II</td>
<td>2 x 10^{-8}</td>
<td>15 x 10^{-4}</td>
<td>5.2</td>
<td>6.7</td>
<td>7.5</td>
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<td>29.0</td>
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<tr>
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<td>4.4</td>
<td>16.5</td>
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<tr>
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<td>4.9</td>
<td>7.4</td>
<td>14.0</td>
<td>38.8</td>
<td>38.8</td>
<td>38.8</td>
<td>38.8</td>
</tr>
<tr>
<td>Mean ± S.E.M.</td>
<td>4.4 ± 0.7</td>
<td>6.2 ± 0.9</td>
<td>12.7±2.7</td>
<td>40.1 ± 6.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>207 I</td>
<td>0.5 x 10^{-8}</td>
<td>25 x 10^{-4}</td>
<td>5.5</td>
<td>5.9</td>
<td>4.7</td>
<td>49.1</td>
<td>49.1</td>
</tr>
<tr>
<td>209 II</td>
<td>4.3</td>
<td>4.6</td>
<td>5.4</td>
<td>54.5</td>
<td>54.5</td>
<td>54.5</td>
<td>54.5</td>
</tr>
<tr>
<td>212 II</td>
<td>4.3</td>
<td>5.2</td>
<td>4.3</td>
<td>45.1</td>
<td>45.1</td>
<td>45.1</td>
<td>45.1</td>
</tr>
<tr>
<td>Mean ± S.E.M.</td>
<td>4.9 ± 0.3</td>
<td>5.2 ± 0.4</td>
<td>4.4 ± 0.1</td>
<td>49.6 ± 2.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>210 I</td>
<td>1 x 10^{-8}</td>
<td>25 x 10^{-4}</td>
<td>5.9</td>
<td>6.4</td>
<td>5.0</td>
<td>47.4</td>
<td>47.4</td>
</tr>
<tr>
<td>212 II</td>
<td>4.0</td>
<td>4.5</td>
<td>5.5</td>
<td>39.4</td>
<td>39.4</td>
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<td>39.4</td>
</tr>
<tr>
<td>215 I</td>
<td>2.7</td>
<td>3.0</td>
<td>9.1</td>
<td>85.1</td>
<td>85.1</td>
<td>85.1</td>
<td>85.1</td>
</tr>
<tr>
<td>Mean ± S.E.M.</td>
<td>4.2 ± 0.9</td>
<td>4.6 ± 1.0</td>
<td>6.5 ± 1.3</td>
<td>57.3 ± 14.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>209 II</td>
<td>2 x 10^{-8}</td>
<td>25 x 10^{-4}</td>
<td>4.9</td>
<td>6.1</td>
<td>17.4</td>
<td>82.2</td>
<td>82.2</td>
</tr>
<tr>
<td>214 II</td>
<td>4.0</td>
<td>5.2</td>
<td>13.9</td>
<td>55.6</td>
<td>55.6</td>
<td>55.6</td>
<td>55.6</td>
</tr>
<tr>
<td>216 I</td>
<td>5.2</td>
<td>6.8</td>
<td>21.0</td>
<td>81.3</td>
<td>81.3</td>
<td>81.3</td>
<td>81.3</td>
</tr>
<tr>
<td>Mean ± S.E.M.</td>
<td>4.7 ± 0.4</td>
<td>6.0 ± 0.5</td>
<td>17.4±2.0</td>
<td>73.0 ± 8.7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$t_{off}$ is the time constant for the rate of decrease of lachesine's occupancy.

$DR_{F}$ is the equilibrium dose ratio produced by the concentration (SL) of lachesine in that experiment.

$DR_{F+SL}$ is the equilibrium dose ratio produced by the concentrations SL and F acting together.

If $DR_{F+SL} = 1 + SL K_{SL} F + K_F$, then $DR_{F+SL} = SL K_{SL} (1 + F K_F) = DR_{F} SL$.

Therefore, if dissociation from the receptors is rate limiting:

$$
t_{off} = \frac{DR_{F}}{DR_{F} + DR_{SL} + DR_{F+SL}} = \frac{1}{K_2}
$$
CONCENTRATION OF PENTYL TEA (M) x10^4

DIAGRAM II.7: The relationship between t<sub>off</sub> DR<sub>F</sub>+SL/(DR<sub>F</sub>+SL-DR<sub>SL</sub>+1) and the concentration of pentyl TEA.

Details
(see also TABLE II.6)
Preparation - intact pieces of ileum
Bathing solution - Tyrode's
Lever - isotonic
Antagonists - lachesine 0.5 x 10<sup>-8</sup>M — X —
1 x 10<sup>-8</sup>M: — △ —
2 x 10<sup>-8</sup>M: — ○ —
— pentyl TEA, concentrations as indicated
Agonist - carbachol
equilibrium dose ratios shown in TABLE II.2, appear to be independent of the concentration of lachesine used. It therefore seemed reasonable to calculate lachesine's occupancy in this way.

In I.2 DR_{SL} and DR_{F+SL} were calculated and DR_{F+SL} was assumed to be equal to DR_{F} + DR_{SL} - 1. In this section DR_{SL} and DR_{F+SL} were determined in each experiment and therefore these values were used in the calculations.

In I.2 the occupancy of the fast antagonist, \( p_F \) was assumed to be \( F \frac{K_F}{1 + p_F} \). In this section the occupancy of the fast antagonist was assumed to be such that \( DR_{F+SL} = DR_F + DR_{SL} - 1 \), \( DR_F \) being equal to \( (DR_{F+SL} - DR_{SL} + 1) \), \( DR_{F+SL} \) and \( DR_{SL} \) being the experimentally determined values.

In I.2 and I.3, it was found that the combined dose ratio produced by pentyl TEA and BTrMe agreed reasonably well with that calculated from the relationship:

\[
DR_{F+SL} = DR_F + DR_{SL} - 1
\]

where \( DR_F = 1 + F \frac{K_F}{1 + F K_F} \)

and \( DR_{SL} = 1 + SL K_{SL} \)

\( K_F = 3.6 \times 10^4 \text{ M}^{-1} \)

\( K_{SL} = 1.5 \times 10^{10} \text{ M}^{-1} \)

However in this section not such good agreement was found between the combined dose ratio of lachesine and pentyl TEA determined experimentally and that calculated as above. In TABLE II.8 the experimentally determined values of \( DR_{F+SL} \) are compared with those calculated as above, and also calculated using the values of \( DR_F \) determined experimentally, TABLE I.19. The experimentally determined values of \( DR_{F+SL} \) are intermediate between the two calculated values.
TABLE II.8: Comparing the experimentally determined values of \( \text{DR}_{F+SL} \) with those calculated from the relationship:

\[
\text{DR}_{F+SL} = \text{DR}_F + \text{DR}_{SL} - 1,
\]

using the values of \( \text{DR}_{SL} \) determined experimentally, (TABLE II.6) together with values of \( \text{DR}_F \), (a) calculated from the relationship:

\[
\text{DR}_F = 1 - F \cdot K_p, \quad K_p = 3.6 \times 10^4 \text{M}^{-1}
\]

(see 1.4), or (b) using the experimentally determined values of \( \text{DR}_F \) indicated in DIAGRAM I.21.

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Conc. of Lachesine (SL) M</th>
<th>Conc. of Pentyl TEA (F) M</th>
<th>Exponentially Determined</th>
<th>Calculated (a)</th>
<th>Calculated (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>206 I</td>
<td>(0.5 \times 10^{-8})</td>
<td>(2.5 \times 10^{-4})</td>
<td>14.0</td>
<td>14.3</td>
<td>14.3</td>
</tr>
<tr>
<td>213 I</td>
<td>(0.5 \times 10^{-8})</td>
<td>(2.5 \times 10^{-4})</td>
<td>14.1</td>
<td>15.4</td>
<td>15.4</td>
</tr>
<tr>
<td>215 I</td>
<td>(1 \times 10^{-8})</td>
<td>(2.5 \times 10^{-4})</td>
<td>17.8</td>
<td>13.7</td>
<td>13.7</td>
</tr>
<tr>
<td>211 I</td>
<td>(1 \times 10^{-8})</td>
<td>(2.5 \times 10^{-4})</td>
<td>17.7</td>
<td>15.8</td>
<td>15.8</td>
</tr>
<tr>
<td>214 I</td>
<td>(2 \times 10^{-8})</td>
<td>(2.5 \times 10^{-4})</td>
<td>18.3</td>
<td>18.3</td>
<td>18.3</td>
</tr>
<tr>
<td>215 I</td>
<td>(2 \times 10^{-8})</td>
<td>(2.5 \times 10^{-4})</td>
<td>21.6</td>
<td>18.1</td>
<td>18.1</td>
</tr>
<tr>
<td>211 I</td>
<td>(0.5 \times 10^{-8})</td>
<td>(15 \times 10^{-4})</td>
<td>47.4</td>
<td>58.3</td>
<td>47.3</td>
</tr>
<tr>
<td>213 I</td>
<td>(1 \times 10^{-8})</td>
<td>(15 \times 10^{-4})</td>
<td>59.7</td>
<td>59.0</td>
<td>58.0</td>
</tr>
<tr>
<td>215 I</td>
<td>(1 \times 10^{-8})</td>
<td>(15 \times 10^{-4})</td>
<td>59.8</td>
<td>58.7</td>
<td>47.7</td>
</tr>
<tr>
<td>210 I</td>
<td>(2 \times 10^{-8})</td>
<td>(15 \times 10^{-4})</td>
<td>35.2</td>
<td>59.0</td>
<td>58.0</td>
</tr>
<tr>
<td>212 I</td>
<td>(2 \times 10^{-8})</td>
<td>(15 \times 10^{-4})</td>
<td>38.7</td>
<td>59.5</td>
<td>58.5</td>
</tr>
<tr>
<td>214 I</td>
<td>(2 \times 10^{-8})</td>
<td>(15 \times 10^{-4})</td>
<td>57.0</td>
<td>63.5</td>
<td>52.5</td>
</tr>
<tr>
<td>210 I</td>
<td>(0.5 \times 10^{-8})</td>
<td>(25 \times 10^{-4})</td>
<td>49.1</td>
<td>94.7</td>
<td>55.7</td>
</tr>
<tr>
<td>209 II</td>
<td>(0.5 \times 10^{-8})</td>
<td>(25 \times 10^{-4})</td>
<td>54.5</td>
<td>94.3</td>
<td>55.3</td>
</tr>
<tr>
<td>212 II</td>
<td>(1 \times 10^{-8})</td>
<td>(25 \times 10^{-4})</td>
<td>45.1</td>
<td>94.3</td>
<td>55.3</td>
</tr>
<tr>
<td>210 I</td>
<td>(1 \times 10^{-8})</td>
<td>(25 \times 10^{-4})</td>
<td>47.4</td>
<td>95.0</td>
<td>56.0</td>
</tr>
<tr>
<td>212 I</td>
<td>(1 \times 10^{-8})</td>
<td>(25 \times 10^{-4})</td>
<td>39.4</td>
<td>95.5</td>
<td>56.5</td>
</tr>
<tr>
<td>215 I</td>
<td>(1 \times 10^{-8})</td>
<td>(25 \times 10^{-4})</td>
<td>85.1</td>
<td>99.1</td>
<td>60.1</td>
</tr>
<tr>
<td>209 II</td>
<td>(2 \times 10^{-8})</td>
<td>(25 \times 10^{-4})</td>
<td>82.2</td>
<td>107.4</td>
<td>68.4</td>
</tr>
<tr>
<td>214 II</td>
<td>(2 \times 10^{-8})</td>
<td>(25 \times 10^{-4})</td>
<td>55.8</td>
<td>103.9</td>
<td>64.9</td>
</tr>
<tr>
<td>216 I</td>
<td>(2 \times 10^{-8})</td>
<td>(25 \times 10^{-4})</td>
<td>81.3</td>
<td>111.0</td>
<td>72.0</td>
</tr>
</tbody>
</table>

Details
(see also TABLE II.6)
Preparation - intact pieces of ileum
Bathing solution - Tyrode's
Lever - isotonic
Antagonists - Lachesine (SL)
Agonist - carbachol
SUMMARY OF PART II

The kinetics of lachesine do not appear to be limited by the rate at which it interacts with the receptors, the following observations being incompatible with the predictions of the interaction limited model:

1. Occupancy does not appear to change exponentially during the onset and offset of antagonism.

2. The ratio $t_{off}/t_{on}$ is not equal to $DR_{\infty}$ when high concentrations of lachesine are examined, the difference appearing to increase as the concentration of lachesine is increased.

3. The rate of decrease in lachesine's occupancy appears to be accelerated by the superimposition of pentyl TEA; values of $t_{off}(DR_{\infty})/(DR_{\infty} - DR_{SL})$, which would be equal to $1/k_2$ if interaction was rate-limiting, decreased as the concentration of the fast antagonist increased. These values are much lower than the apparent value of $1/k_2$ determined from the rates of offset.

Thus the kinetic behaviour of lachesine, like that of BTrMe, is not compatible with the predictions of the interaction limited model.

Similarly the possibility cannot be ruled out that the rate of offset of lachesine is dissociation limited following the superimposition of very high concentrations of pentyl TEA.
DISCUSSION

When Paton & Rang (1965) investigated the kinetics of lachesine they found that recovery from lachesine's antagonism took place with a time constant of 4.5 minutes. This is considerably faster than any of the rates observed in II,1 although the value of the affinity constant they calculated from their equilibrium dose ratios is similar to that found here. In addition they found that occupancy appeared to change exponentially with time and also that toff/ton agreed with the equilibrium dose ratio. Thus although the kinetics of lachesine observed in this section appear to be inconsistent with the interaction limited model, those observed by Paton & Rang (1965) are not.

There is also a striking similarity between the time constant of recovery observed by Paton & Rang (1965) and the limiting value of apparent 1/k2 indicated in DIAGRAM II.7. It is therefore possible that although the kinetics of antagonists are access limited when certain experimental conditions are used, they may be interaction limited when other experimental conditions are used.

There are several notable differences between the procedure used by Paton & Rang (1965) and that used in I and II. They used longitudinal muscle strips taken from animals over 500g in weight. These strips were suspended in a Krebs solution and the responses, produced by acetylcholine, were recorded with an auxotonic lever. This contrasts with the conditions in I and II in which intact pieces of ileum were taken from animals usually much less than 500g in weight. These pieces of ileum were suspended in Tyrode's solution and the responses, produced by carbachol, were recorded with an isotonic lever. The differences between the observations of this study and those of Paton & Rang (1965) may therefore be associated with the different procedures used.

The kinetics of ETMMe and lachesine were therefore investigated under a variety of experimental conditions.
PART III

In the course of this study the possible effect of experimental method on the kinetics of BTrMe and lachesine was investigated. In III.1-5 comparisons are made between muscle strips and intact pieces of ileum, between using an isometric transducer and an isotonic lever, between using pentyl TMA and carbachol, and lastly between using Krebs solution and Tyrode's solution. These studies were undertaken for several reasons:

Firstly they were undertaken to investigate to what extent the discrepancies found in I and II between the kinetics of lachesine and BTrMe and the predictions of the interaction limited model may be due to the experimental method used.

Secondly, they might provide an explanation for the perplexing variation in the rates of antagonism observed by different groups of workers. For instance the discrepancy between the kinetics of lachesine observed by Paton & Rang (1965) and that found in II.

Lastly, any effects of altering the experimental method on the kinetics of antagonism might provide insight into the type of access limitation.

In the initial studies, III.1 and III.2, BTrMe was used. In the subsequent studies, III.3-5, lachesine was used rather than BTrMe because its faster speed enabled more experiments to be done in a given time and also would decrease the effect of the tissue sensitivity changing with time. III.1-5 are discussed together following III.5.

The rate of offset of lachesine on superimposition of octyl TMA or Ph₂AObEt was also investigated, III.6, to determine whether the limiting value of $D_{\text{off}}/(D_{\text{E-P}} + t_{\text{off}})$ was different when either of these fast antagonists were superimposed as compared with when pentyl TEA was superimposed. If the limiting value is equal to $1/k_2$ it might
not be expected to vary.

In III.7 experiments are described in which the rate of onset and offset of BTrMe was followed after the tissue had been treated with an irreversible antagonist.

In III.8 experiments are described in which the rate of onset and offset of lachesine was followed using hexyl TMA rather than carbachol.
III.1 THE KINETICS OF BTMë USING MUSCLE STRIPS AND AN ISOMETRIC TRANSDUCER

Procedure and Results

The experiments of I.1 were repeated using the same method as described in I.1, except that longitudinal muscle strips were used instead of intact pieces of ileum, and also an isometric transducer was used to record the responses rather than an isotonic lever.

These experiments are shown in DIAGRAMS III.1-3, together with the results of I.1 for comparison. The points are plotted as described on P.60. The time constants for onset and offset, determined for each experiment as in I.1, are shown in TABLE III.4.

When muscle strips were used together with an isometric transducer, (compared with using intact pieces of ileum and an isotonic lever):

1. the rates of both onset and offset were slower,
2. the rate of offset did not appear to depend on the concentration of BTMë used, and
3. for onset, the relationship between log occupancy and time appeared to be more linearly related.

Nevertheless, using muscle strips and an isometric transducer, access must still be the rate-limiting stage because:

1. the values of $t_{off}$ are larger than the limiting value of $t_{off} / DR_{\infty}$ found in I.2,
2. the ratio $t_{off} / t_{on}$ was not equal to $DR_{\infty}$ when high concentrations of BTMë were used, the difference increasing as the concentration of BTMë increased.

These differences can not be accounted for by a change in the equilibrium dose ratio. Also there was no evidence of non-equilibrium effects in these experiments. In addition, although the experiments
DIAGRAM III.1: The kinetics of onset and offset of $10 \times 10^{-10}$ M BTrMe - comparing the rates found using longitudinal muscle strips and an isometric transducer with those using intact pieces of ileum and an isotonic lever, (from I.1)

Details
(see also TABLES I.3 and III.4)
Preparation & Lever - intact pieces of ileum and an isotonic lever
\begin{itemize}
  \item $n = 6$ Mean DR$\infty$ S.E.M. = 15.7 $\pm$ 0.9
  \item muscle strips and an isometric transducer
  \item $n = 3$ Mean DR$\infty$ S.E.M. = 24.6 $\pm$ 7.5
\end{itemize}

Bathing solution - Tyrode's
Antagonist - BTrMe $10 \times 10^{-10}$ M
Agonist - carbachol

For onset mean values of $(p_\infty - p_C) \pm$ S.E.M. are plotted on a log scale against time
For offset mean values of $(p_C) \pm$ S.E.M. are plotted on a log scale against time
Diagram III.2: The kinetics of onset and offset of $20 \times 10^{-10}$ M BTrMe - comparing the rates found using longitudinal muscle strips and an isometric transducer with those found using intact pieces of ileum and an isotonic lever, (from I.1)

For onset, mean values of $(p_{oo} - p_{t}) \pm S.E.M.$ are plotted on a log scale against time.

For offset, mean values of $(p_{t}) \pm S.E.M.$ are plotted on a log scale against time.

Details
(see also TABLES I.3 & III.4)
Preparation & Lever - intact pieces of ileum and an isotonic lever
  - muscle strips and an isometric transducer
  - $n = 4$  Mean $DR_{oo} \pm S.E.M. = 36.0 \pm 3.6$
  - $n = 4$  Mean $DR_{oo} \pm S.E.M. = 38.1 \pm 4.7$

Bathing solution - Tyrode's
Agonist - BTrMe $20 \times 10^{-10}$ M
Agonist - carbachol
DIAGRAM III.3: The kinetics of onset and offset of 40 x 10^{-10} M BTrMe — comparing the rates found using longitudinal muscle strips and an isometric transducer with those found using intact pieces of ileum and an isotonic lever, (from I:1).

For onset, mean values of (p_{oo} - p_t) \pm S.E.M. are plotted on a log scale against time.

For offset, mean values of (p_t) \pm S.E.M. are plotted on a log scale against time.

Details (see also TABLES I.3 & III.4)

Preparation & Lever — intact pieces of ileum and an isotonic lever

- X — n = 3  Mean DR_{oo} \pm S.E.M. = 59.7 \pm 2.4
- o — n = 4  Mean DR_{oo} \pm S.E.M. = 76.5 \pm 15.3

Antagonist — BTrMe
Agonist — carbachol
**TABLE III.4: The kinetics of onset and offset of BTrMe**

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Concentration of Antagonist C (M)</th>
<th>Time Constant for Onset (mins)</th>
<th>Time Constant for Offset (mins)</th>
<th>t_{off} / t_{on}</th>
<th>Equilibrium Dose Ratio D_{Eo}</th>
</tr>
</thead>
<tbody>
<tr>
<td>178 II</td>
<td>$10 \times 10^{-10}$</td>
<td>26</td>
<td>931</td>
<td>35.9</td>
<td>39</td>
</tr>
<tr>
<td>178 I</td>
<td></td>
<td>56</td>
<td>412</td>
<td>7</td>
<td>17</td>
</tr>
<tr>
<td>180 II</td>
<td></td>
<td>18</td>
<td>391</td>
<td>21</td>
<td>16</td>
</tr>
<tr>
<td>Mean ± S.E.M.</td>
<td>33 ± 12</td>
<td>578 ± 177</td>
<td></td>
<td>25 ± 8</td>
<td></td>
</tr>
<tr>
<td>175 I</td>
<td>$20 \times 10^{-10}$</td>
<td>14</td>
<td>238</td>
<td>17</td>
<td>47</td>
</tr>
<tr>
<td>176 I</td>
<td></td>
<td>28</td>
<td>519</td>
<td>19</td>
<td>34</td>
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<td>177 II</td>
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<td>637</td>
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<td>181 I</td>
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<td>182 II</td>
<td></td>
<td></td>
<td>567</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± S.E.M.</td>
<td>20 ± 3</td>
<td>509 ± 60</td>
<td></td>
<td>38 ± 5</td>
<td></td>
</tr>
<tr>
<td>174 I</td>
<td>$40 \times 10^{-10}$</td>
<td>9</td>
<td>471</td>
<td>52</td>
<td>33</td>
</tr>
<tr>
<td>174 II</td>
<td></td>
<td>6</td>
<td>425</td>
<td>71</td>
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</tr>
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<td>179 I</td>
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<td></td>
<td>22</td>
<td>706</td>
<td>32</td>
<td>91</td>
</tr>
<tr>
<td>Mean ± S.E.M.</td>
<td>13 ± 4</td>
<td>502 ± 69</td>
<td></td>
<td>77 ± 15</td>
<td></td>
</tr>
</tbody>
</table>

**Details**
- Preparation - longitudinal muscle strips
- Bathing solution - Tyrode's
- Lever - isometric
- Antagonist - BTrMe
- Agonist - carbachol
were carried out in two successive groups, it is unlikely that this could account for the differences; For instance the onset and offset of $10 \times 10^{-10} \text{M BTrMe}$ was followed in six experiments using the alternating technique described in I.1. However 4 of these experiments were done over a year before the other two. DIAGRAM III.5 compares these two groups of experiments. (In I.1. these experiments were grouped together)
For onset, mean values of \((p_\infty - p_L) \pm S.E.M.\) are plotted on a log scale against time.

For offset, mean values of \((p_L) \pm S.E.M.\) are plotted on a log scale against time.

**Details**

Preparation - intact pieces of ileum
Bathing solution - Tyrode's
Lever - isotonic
Antagonist - BTrMe \(10 \times 10^{-10}\) M
Agonist - carbachol

\[ n = 4 \quad \text{Exp. Nos. 37, 38, 39, 40} \quad \text{Mean DR}_{\infty} \pm S.E.M. = 14.5 \pm 1.3 \]

\[ n = 3 \quad \text{Exp. Nos. 167 I, 169 I, 170 II} \quad \text{Mean DR}_{\infty} \pm S.E.M. = 16.8 \pm 1.2 \]
III.2 THE KINETICS OF BTrMe USING THE AGONIST PENTYL TMA

Procedure & Results

The rate of onset and offset of $10 \times 10^{-10}$M BTrMe was followed using the alternating technique described in I.1, except that pentyl TMA (pentyl trimethylammonium), was used instead of carbachol. In DIAGRAM III.6 these experiments are compared with those of I.1. (The points are plotted as described on p. 60.)

The rates of onset and offset of BTrMe do not appear to be markedly affected by whether carbachol or pentyl TMA was used. However the variation was larger when pentyl TMA was used. This may be associated with the larger variation in $D_R^{\infty}$. Non-equilibrium effects might cause a larger variation in $D_R^{\infty}$ but there was no evidence that the apparent kinetics using pentyl TMA were effected by non-equilibrium effects, and such effects would not be expected when the antagonist concentration is $10 \times 10^{-10}$M, (see I.4).

The difference between the equilibrium dose ratios is discussed in the Appendix.
The kinetics of onset and offset of $10 \times 10^{-10}$ M BTrMe comparing the rate observed using pentyl TMA with that observed using carbachol.

For onset, mean values of $(p_\infty - p_t) \pm \text{S.E.M.}$ are plotted on a log scale against time.

For offset, mean values of $(p_t) \pm \text{S.E.M.}$ are plotted on a log scale against time.

**Details**
- Preparations: intact pieces of ileum
- Bathing solution: Tyrode's
- Lever: isotonic
- Antagonist: BTrMe $10 \times 10^{-10}$ M
- Agonist: carbachol

$p_\infty$ values:
- Mean $p_\infty \pm \text{S.E.M.}$ for $n=3$ (Exp. Nos. 37, 38, 39) = $14.5 \pm 1.3$
- Mean $p_\infty \pm \text{S.E.M.}$ for $n=4$ (Exp. Nos. 37, 38, 39, 40) = $19.0 \pm 8.0$
III.3: THE KINETICS OF LACHESINE USING MUSCLE STRIPS OR INTACT PIECES OF ILEUM, AND PENTYL TMA OR CARBACHOL

Procedure and Results

The rate of onset and offset of $1 \times 10^{-8} \text{M}$ lachesine was followed as in II.1 except that longitudinal muscle strips were used instead of intact pieces of ileum. In addition the kinetics were followed using pentyl TMA instead of carbachol.

These experiments are shown in DIAGRAM III.7, together with the results given in II.1 for comparison. The points are plotted as described on P.60. In particular the points corresponding to alternate responses are not plotted during offset.

As in II.1 time constants corresponding to the initial rate of change were calculated for each experiment from the first three responses after the antagonist was added or removed. These values are shown in TABLE III.8.

The rate of onset does not appear to be appreciably affected by which preparation or agonist was used. However the rate of offset using carbachol and intact pieces of ileum appears to be faster than in the other conditions. However the fact that 5 of the experiments of this group, as shown in TABLE III.8, were done at a later date than the others, suggests that some other factor might be involved in this difference.

The difference between the equilibrium dose ratios is discussed in the Appendix.
DIAGRAM III.7: The kinetics of onset and offset of $1 \times 10^{-8} \text{M}$ lachesine - using intact pieces of ileum or muscle strips, and carbachol or pentyl TMA

For onset, mean values of $(p_{oo} - p_c) \pm \text{S.E.M.}$ are plotted on a log scale against time.
For offset, mean values of $(p_c) \pm \text{S.E.M.}$ are plotted on a log scale against time.

Details
Preparation & Agonist - intact pieces of ileum and carbachol
\[ n = 7 \quad \text{Mean DR}_{oo} \pm \text{S.E.M.} = 7.4 \pm 0.8 \]
- intact pieces of ileum and pentyl TMA
\[ n = 2 \quad \text{Mean DR}_{oo} \pm \text{S.E.M.} = 9.9 \pm 0.6 \]
- muscle strips and carbachol
\[ n = 7 \quad \text{Mean DR}_{oo} \pm \text{S.E.M.} = 8.9 \pm 0.3 \]
- muscle strips and pentyl TMA
\[ n = 3 \quad \text{Mean DR}_{oo} \pm \text{S.E.M.} = 10.5 \pm 0.1 \]

Bathing solution - Tyrode's
Lever - isotonic
Antagonist - lachesine $1 \times 10^{-8} \text{M}$
### TABLE III.8: The rate of onset and offset of $1 \times 10^{-8}$M lachesine

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>$t_{on}'$ mins</th>
<th>$t_{off}'$ mins</th>
<th>$\text{DR}_{\infty}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intact pieces of ileum and carbachol</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>81 I</td>
<td>4.4</td>
<td>29.3</td>
<td>9.4</td>
</tr>
<tr>
<td>82 I</td>
<td>4.7</td>
<td>26.6</td>
<td>9.3</td>
</tr>
<tr>
<td>205 II</td>
<td>6.7</td>
<td>19.9</td>
<td>5.7</td>
</tr>
<tr>
<td>210 II</td>
<td>5.6</td>
<td>10.7</td>
<td>5.0</td>
</tr>
<tr>
<td>211 II</td>
<td>6.2</td>
<td>13.0</td>
<td>6.8</td>
</tr>
<tr>
<td>212 I</td>
<td>4.9</td>
<td>13.7</td>
<td>5.9</td>
</tr>
<tr>
<td>214 I</td>
<td>7.5</td>
<td>43.1</td>
<td>9.5</td>
</tr>
<tr>
<td>Mean ± S.E.M.</td>
<td>5.7±0.4</td>
<td>22.3±4.4</td>
<td>7.4±0.8</td>
</tr>
<tr>
<td><strong>Intact pieces of ileum and pentyl TMA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>91 I</td>
<td>7.6</td>
<td>29.3</td>
<td>10.5</td>
</tr>
<tr>
<td>92 I</td>
<td>4.8</td>
<td>28.9</td>
<td>9.3</td>
</tr>
<tr>
<td>Mean ± S.E.M.</td>
<td>6.2±1.4</td>
<td>29.1±0.2</td>
<td>9.9±0.6</td>
</tr>
<tr>
<td><strong>Longitudinal muscle strips and carbachol</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>84 I</td>
<td>5.1</td>
<td>18.0</td>
<td>9.0</td>
</tr>
<tr>
<td>85 I</td>
<td>6.5</td>
<td>24.7</td>
<td>8.2</td>
</tr>
<tr>
<td>85 II</td>
<td>9.7</td>
<td>60.0</td>
<td>8.4</td>
</tr>
<tr>
<td>86 I</td>
<td>3.7</td>
<td>35.6</td>
<td>9.0</td>
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<tr>
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<td>2.8</td>
<td>33.5</td>
<td>9.9</td>
</tr>
<tr>
<td>87 I</td>
<td>3.6</td>
<td>27.6</td>
<td>9.9</td>
</tr>
<tr>
<td>88 I</td>
<td>3.2</td>
<td>17.3</td>
<td>7.8</td>
</tr>
<tr>
<td>Mean ± S.E.M.</td>
<td>4.9±0.9</td>
<td>30.9±5.5</td>
<td>8.9±0.3</td>
</tr>
<tr>
<td><strong>Longitudinal muscle strips and pentyl TMA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>94 I</td>
<td>3.6</td>
<td>29.7</td>
<td>10.6</td>
</tr>
<tr>
<td>95 I</td>
<td>2.4</td>
<td>20.7</td>
<td>10.4</td>
</tr>
<tr>
<td>96 I</td>
<td>3.8</td>
<td>25.9</td>
<td>10.5</td>
</tr>
<tr>
<td>Mean ± S.E.M.</td>
<td>3.3±0.4</td>
<td>25.4±2.0</td>
<td>10.5±0.1</td>
</tr>
</tbody>
</table>

$\text{DR}_{\infty}$ is the equilibrium dose ratio

$t_{on}'$ and $t_{off}'$ were calculated from the first three responses after the antagonist was added or removed

* These results are also given in TABLE II.2

**Details**
- Preparation: intact pieces of ileum or muscle strips
- Bathing solution: Tyrode's
- Lever: isotonic
- Antagonist: $1 \times 10^{-8}$M lachesine
- Agonists: carbachol or pentyl TMA
III.4: THE KINETICS OF LACHESINE FOLLOWED USING A VARIETY OF METHODS

Procedure and Results

The rate of onset and offset of $2 \times 10^{-8}$M lachesine was followed using the four combinations of lever and preparation:

- intact pieces of ileum and an isotonic lever,
- intact pieces of ileum and an isometric transducer,
- muscle strips and an isotonic lever,
- muscle strips and an isometric transducer.

At the same time the rate of offset of lachesine on superimposition of $15 \times 10^{-4}$M pentyl TEA was investigated under the 4 conditions to see whether any change in this rate of offset corresponded to any similar change in the rate of onset and recovery from antagonism. This concentration of pentyl TEA was used rather than $25 \times 10^{-6}$M because it would be expected to reflect changes in the access-limitation more than higher concentrations would, as shown in DIAGRAM II.7.

In each experiment two preparations were used: with the first, the onset of lachesine was followed as in II, and then either the offset by removal of the lachesine or offset by superimposition of pentyl TEA. (During this time the second piece of tissue was equilibrating with $2 \times 10^{-8}$M lachesine in a beaker). The second piece of tissue was then set up and when it had settled down was then used to follow either the rate of offset on removal of the lachesine or the rate of offset on superimposition of pentyl TEA. In one half of the experiments one order was used, and in the other half the reverse order was used. (No evidence was found that the kinetics of antagonism were effected by the order)

$D_{3L}^{R}$ was determined from the first piece of tissue and this value was used for the calculations of that experiment. As in II
the interaction between lachesine and pentyl TEA was followed until an equilibrium was established.

Time constants were calculated in each experiment as described in II. These are shown in TABLE III.9, together with the values of DR_{SL} observed in each experiment.

4 of the experiments in which intact pieces of ileum were used with an isotonic lever, (217 I, 220 II, 223 II, 227 I), were performed in a block with the three other groups, i.e. 4 groups with 4 observations in each. These results were examined by means of a variance analysis as shown below. (As a value of $t_{off}$ was not obtained in Exp. No. 227 I, the value obtained in 214 II is included into that group).

### Analysis of Variance of Dose Ratio

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>d.f.</th>
<th>S.S.</th>
<th>M.S.</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Levers</td>
<td>1</td>
<td>27.04</td>
<td>27.04</td>
<td>3.469</td>
<td>P &gt; .05</td>
</tr>
<tr>
<td>Between Preparations</td>
<td>1</td>
<td>1.21</td>
<td>1.21</td>
<td>0.155</td>
<td>P &gt; .2</td>
</tr>
<tr>
<td>Interaction</td>
<td>1</td>
<td>0.16</td>
<td>0.16</td>
<td>0.021</td>
<td>P &gt; .1</td>
</tr>
<tr>
<td>Error</td>
<td>12</td>
<td>93.53</td>
<td>7.799</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>d.f. - degrees of freedom</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.S. - Sum of Squares</td>
</tr>
<tr>
<td>M.S. - Mean Square</td>
</tr>
<tr>
<td>F - Variance Ratio</td>
</tr>
<tr>
<td>P - the probability of F being greater or equal to the observed value</td>
</tr>
<tr>
<td>P* - the probability of F being less or equal to the observed value</td>
</tr>
</tbody>
</table>

### Analysis of variance of $t_{on}$

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>d.f.</th>
<th>S.S.</th>
<th>M.S.</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Levers</td>
<td>1</td>
<td>0.913</td>
<td>0.913</td>
<td>0.077</td>
<td>P &gt; .2</td>
</tr>
<tr>
<td>Between Preparations</td>
<td>1</td>
<td>37.22</td>
<td>37.22</td>
<td>3.154</td>
<td>P &gt; .2</td>
</tr>
<tr>
<td>Interaction</td>
<td>1</td>
<td>1.103</td>
<td>1.103</td>
<td>0.0934</td>
<td>P &gt; .2</td>
</tr>
<tr>
<td>Error</td>
<td>12</td>
<td>141.625</td>
<td>11.802</td>
<td></td>
<td></td>
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</tbody>
</table>
TABLE III.9: The kinetics of $2 \times 10^{-8}$M lachesine

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>$t_{on}$ mins</th>
<th>$t_{off}$ mins</th>
<th>$t_{off} \frac{DR_{P+SL}}{DR_{P+SL} - DR_{SL}}$</th>
<th>DR$_{SL}$</th>
<th>DR$_{P+SL}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intact pieces of ileum and an isotonic lever</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>204 I</td>
<td>3.9</td>
<td>35.8</td>
<td>17.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>206 II</td>
<td>3.7</td>
<td>8.3</td>
<td>11.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>208 II</td>
<td>2.5</td>
<td>11.8</td>
<td>8.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>209 I</td>
<td>2.4</td>
<td>30.9</td>
<td>17.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>210 II</td>
<td>6.6</td>
<td>15.0</td>
<td>6.7</td>
<td>7.5</td>
<td>29.0</td>
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<tr>
<td>211 II</td>
<td>2.8</td>
<td>28.5</td>
<td>13.2</td>
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<tr>
<td>213 II</td>
<td>3.7</td>
<td>37.7</td>
<td>4.4</td>
<td>16.3</td>
<td>52.4</td>
</tr>
<tr>
<td>214 II</td>
<td>2.9</td>
<td>33.2</td>
<td>7.4</td>
<td>14.0</td>
<td>38.8</td>
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<tr>
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<td>4.5</td>
<td>16.3</td>
<td>2.5</td>
<td>12.0</td>
<td>35.4</td>
</tr>
<tr>
<td>219 I</td>
<td>3.8</td>
<td>17.8</td>
<td>5.8</td>
<td>18.2</td>
<td>58.9</td>
</tr>
<tr>
<td>220 II</td>
<td>3.0</td>
<td>14.8</td>
<td>14.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>222 II</td>
<td>4.8</td>
<td>20.8</td>
<td>16.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>223 I</td>
<td>0.4</td>
<td>22.2</td>
<td>15.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>225 II</td>
<td>2.7</td>
<td>26.3</td>
<td>6.5</td>
<td>22.9</td>
<td>57.7</td>
</tr>
<tr>
<td>227 I</td>
<td>0.4</td>
<td>49.3</td>
<td>19.4</td>
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<td></td>
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<tr>
<td>231 II</td>
<td>3.0</td>
<td>13.8</td>
<td>14.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean±S.E.M. 3.7±0.3</td>
<td>25.6±3.0</td>
<td>5.6±0.7</td>
<td>14.9±1.0</td>
<td>45.4±5.1</td>
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</tr>
<tr>
<td><strong>Intact pieces of ileum and an isometric lever</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>218 I</td>
<td>5.1</td>
<td>67.0</td>
<td>10.1</td>
<td>13.8</td>
<td>59.3</td>
</tr>
<tr>
<td>221 I</td>
<td>3.1</td>
<td>21.3</td>
<td>6.9</td>
<td>17.3</td>
<td>57.1</td>
</tr>
<tr>
<td>224 I</td>
<td>4.9</td>
<td>40.9</td>
<td>5.2</td>
<td>16.2</td>
<td>63.9</td>
</tr>
<tr>
<td>226 I</td>
<td>3.0</td>
<td>32.4</td>
<td>9.0</td>
<td>15.6</td>
<td>57.1</td>
</tr>
<tr>
<td>Mean±S.E.M. 4.0±0.6</td>
<td>40.4±9.7</td>
<td>7.8±1.1</td>
<td>15.7±0.7</td>
<td>59.4±1.6</td>
<td></td>
</tr>
<tr>
<td><strong>Muscle strips and an isotonic lever</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>217 II</td>
<td>7.7</td>
<td>15.5</td>
<td>7.1</td>
<td>15.3</td>
<td>48.4</td>
</tr>
<tr>
<td>222 I</td>
<td>11.3</td>
<td>43.5</td>
<td>2.4</td>
<td>19.8</td>
<td>76.2</td>
</tr>
<tr>
<td>225 I</td>
<td>8.9</td>
<td>35.0</td>
<td>4.4</td>
<td>17.7</td>
<td>61.4</td>
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<tr>
<td>228 I</td>
<td>2.3</td>
<td>41.6</td>
<td>3.1</td>
<td>18.5</td>
<td>45.7</td>
</tr>
<tr>
<td>Mean±S.E.M. 7.6±1.9</td>
<td>33.9±6.4</td>
<td>4.3±1.0</td>
<td>17.8±0.9</td>
<td>57.9±7.0</td>
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<td><strong>Muscle strips and an isometric lever</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>218 II</td>
<td>4.5</td>
<td>19.0</td>
<td>14.6</td>
<td>12.2</td>
<td>52.0</td>
</tr>
<tr>
<td>221 I</td>
<td>4.3</td>
<td>26.4</td>
<td>6.1</td>
<td>16.9</td>
<td>44.4</td>
</tr>
<tr>
<td>224 I</td>
<td>14.8</td>
<td>26.6</td>
<td>4.4</td>
<td>14.2</td>
<td>66.6</td>
</tr>
<tr>
<td>226 I</td>
<td>2.6</td>
<td>75.0</td>
<td>4.0</td>
<td>16.6</td>
<td>52.0</td>
</tr>
<tr>
<td>Mean±S.E.M. 6.6±2.8</td>
<td>36.8±12.9</td>
<td>7.3±2.5</td>
<td>15.0±1.1</td>
<td>53.8±4.6</td>
<td></td>
</tr>
</tbody>
</table>

DR$_{SL}$ is the equilibrium dose ratio produced by lachesine

DR$_{P+SL}$ is the dose ratio produced by lachesine and pentyl TEA together.

$t_{on}$ and $t_{off}$ were calculated from the first three responses after the antagonist was added or removed.

t$_{off}$, the time constant for the rate of decrease of lachesine's occupancy on superimposing $15 \times 10^{-8}$M pentyl TEA, was also calculated from the first three responses after the pentyl TEA was superimposed.

This table includes results, shown * which were obtained as described in XI.

**Details**

Preparation - intact pieces of ileum or muscle strips, as indicated.

Leve - isotonic or isometric, as indicated.

Bathing solution - Tyrode's.

Antagonist - carbachol.

BRot is the equilibrium dose ratio produced by lachesine.

OR is the dose ratio produced by lachesine and pentyl TEA together.

$F_{on} + SL$ and $t_{on}$ were calculated from the first three responses after the antagonist was added or removed.

$F_{off}$, the time constant for the rate of decrease of lachesine's occupancy on superimposing $15 \times 10^{-8}$M pentyl TEA, was also calculated from the first three responses after the pentyl TEA was superimposed.
Analysis of variance of $t_{\text{off}^1}$

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>d.f.</th>
<th>S.S.</th>
<th>M.S.</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Levers</td>
<td>1</td>
<td>117.723</td>
<td>117.723</td>
<td>0.324</td>
<td>$P^* &gt; .2$</td>
</tr>
<tr>
<td>Between Preparations</td>
<td>1</td>
<td>4.623</td>
<td>4.623</td>
<td>0.013</td>
<td>$.1 &gt; P^* &gt; .05$</td>
</tr>
<tr>
<td>Interaction</td>
<td>1</td>
<td>26.523</td>
<td>26.523</td>
<td>0.073</td>
<td>$P^* &gt; .2$</td>
</tr>
<tr>
<td>Error</td>
<td>12</td>
<td>4353.336</td>
<td>362.778</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Analysis of variance of $t_{\text{off}^1} \frac{\text{DR}_F + \text{SL}}{\text{DR}_F + \text{SL} - \text{DR}_S + 1}$

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>d.f.</th>
<th>S.S.</th>
<th>M.S.</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Levers</td>
<td>1</td>
<td>27.826</td>
<td>27.826</td>
<td>2.906</td>
<td>P &gt; .2</td>
</tr>
<tr>
<td>Between Preparations</td>
<td>1</td>
<td>3.331</td>
<td>3.331</td>
<td>0.348</td>
<td>P* &gt; .2</td>
</tr>
<tr>
<td>Interaction</td>
<td>1</td>
<td>0.601</td>
<td>0.601</td>
<td>0.063</td>
<td>$.2 &gt; P^* &gt; .1$</td>
</tr>
<tr>
<td>Error</td>
<td>12</td>
<td>114.908</td>
<td>9.576</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

These tests do not indicate any significant differences ($P < 0.05$).

As the values of $t_{\text{on}^1}$, $t_{\text{off}^1}$ and $t_{\text{off}^1}$ were calculated from the first three responses after the antagonist was added or removed, they do not compare the overall rates. Such tests would therefore not detect differences between levers and preparations which resulted in a change in the curvature of the relationship between log occupancy and time.

In DIAGRAMS III.10-12 longitudinal muscle strips are compared with intact pieces of ileum. In DIAGRAMS III.13-15 those experiments in which an isometric transducer was used are compared with those in which an isotonic lever was used. (The points are plotted as described on p.60)

As shown, for instance in DIAGRAM III.11, there do seem to be differences between the curvature of the plots. In this instance the curvature of the offset plot appears to be less, i.e. more
DIAGRAM III.10: The kinetics of onset and offset of $2 \times 10^{-8} \text{M}$ lachesine - comparing the use of longitudinal muscle strips with the use of intact pieces of ileum (using an isotonic lever).

For onset, mean values of $(p_{\infty} - p_t) \pm S.E.M.$ are plotted on a log scale against time.

For offset, mean values of $(p_t) \pm S.E.M.$ are plotted on a log scale against time.

Details
(see also TABLE III.9)

Preparation - intact pieces of ileum
- muscle strips

$\text{Bathing solution - Tyrode's}$
$\text{Lever - isotonic}$
$\text{Antagonist - lachesine} 2 \times 10^{-8} \text{M}$
$\text{Agonist - carbachol}$
UNIT III.11: The kinetics of onset and offset of $2 \times 10^{-8}$M lachesine - comparing the use of longitudinal muscle strips with the use of intact pieces of ileum (using an isometric transducer)

For onset, mean values of $(p_{oo} - p_c) \pm S.E.M.$ are plotted on a log scale against time.

For offset, mean values of $(p_c) \pm S.E.M.$ are plotted on a log scale against time.

Details (see also TABLE III.9)

Preparation - intact pieces of ileum: $\times$ n=4 mean $D R = S.E.M. = 15.7 \pm 0.7$
- muscle strips $-$ n=4 mean $D R = S.E.M. = 15.0 \pm 1.1$

Lever - isometric
Bathing solution - Tyrode's
Antagonist - lachesine $2 \times 10^{-8}$M
Agonist - carbachol
Diagram III.12: The rate of offset of lachesine on superimposing pentyl TEA - comparing the use of longitudinal muscle strips with the use of intact pieces of ileum. 

- (a) using an isotonic lever
- (b) using an isometric transducer

Mean values of $(P_t - P_{oo}) \pm$ S.E.M. are plotted on a log scale against time.

Details (see also TABLE III.9)

- Preparation - intact pieces of ileum
  - longitudinal muscle strips

- Lever - (a) isotonic
  - (b) isometric

- Bathing solution - Tyrode's

- Antagonists - $2 \times 10^{-6}$M lachesine
  - $15 \times 10^{-4}$M pentyl TEA

- Agonist - carbachol
Figure III.13: The kinetics of onset and offset of $2 \times 10^{-8}$M lachesine comparing the use of an isometric transducer with the use of an isotonic lever (using intact pieces of ileum)

For onset, mean values of $(p_\infty - p_t) \pm$ S.E.M. are plotted on a log scale against time.

For offset, mean values of $(p_t) \pm$ S.E.M. are plotted on a log scale against time.

Details (see also Table III.9)
- Preparation - intact pieces of ileum
- Bathing solution - Tyrode's
- Lever - isotonic $\n = 16$ mean $D_{R,\infty} \pm$ S.E.M. = $14.9 \pm 1.0$
- isometric $\n = 4$ mean $D_{R,\infty} \pm$ S.E.M. = $15.7 \pm 0.7$

Antagonist - lachesine $2 \times 10^{-8}$M
Agonist - carbachol
DIAGRAM III.14: The kinetics of onset and offset of $2 \times 10^{-8}$ M lachesine comparing the use of an isometric transducer with the use of an isotonic lever (using longitudinal muscle strips)

Details
Preparation - muscle strips
Lever - isotonic $\Delta$ $n = 4$ mean $\text{DR}_{\text{iso}} \pm \text{S.E.M.} = 17.8 \pm 0.9$
- isometric $\bigcirc$ $n = 4$ mean $\text{DR}_{\text{iso}} \pm \text{S.E.M.} = 14.9 \pm 1.1$
Antagonist - lachesine $2 \times 10^{-8}$ M
Agonist - carbachol
Bathing solution - Tyrode's
DIAGRAM III.15: The rate of offset of lachesine on superimposing pentyl TEA - comparing the use of an isometric transducer with the use of an isotonic lever 
(a) using intact pieces of ileum  
(b) using muscle strips

Mean values of $(p_t - p_\infty)$ $\pm$ S.E.M. are plotted on a log scale against time

**Details**
(see also TABLE III.9)

**Preparation** - (a) intact pieces of ileum  
- (b) muscle strips

**Lever** - isotonic $\Delta$  
- isometric $\circ$

**Bathing solution** - Tyrode's

**Antagonists** - $2 \times 10^{-8}$ M lachesine  
- $15 \times 10^{-8}$ M pentyl TEA

**Agonist** - carbachol
nearly linear, when muscle strips were used rather than intact pieces of ileum.

Therefore although the analyses of variance rule out large differences in kinetic behaviour between the 4 groups small differences can not be ruled out particularly in view of the small number of observations in each group.
III.5: THE KINETICS OF LACHESINE WHEN THE PREPARATION USED IS BATHED IN KREBS SOLUTION RATHER THAN TYRODE’S

Procedure and Results

The rate of onset and offset of $2 \times 10^{-6} \text{M}$ lachesine was investigated as described in III.4, but the preparations were bathed in Krebs solution rather than Tyrode’s. An isotonic lever was used in all these experiments and both muscle strips and intact pieces of ileum were used.

The rate of offset of lachesine on superimposition of $15 \times 10^{-4} \text{M}$ pentyl TEA was also investigated as in III.4.

Time constants were calculated in each experiment from the first three responses after the addition or removal of the antagonist and these are shown in TABLE III.16, together with the values of $\text{DR}_{\text{SL}}$ observed in each experiment. These results were examined by means of a variance analysis together with those results used in the analysis of III.4 in which an isotonic lever was used.

### Analysis of Variance of Dose Ratio

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>d.f.</th>
<th>S.S.</th>
<th>M.S.</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Preparations</td>
<td>1</td>
<td>4.623</td>
<td>4.623</td>
<td>0.631</td>
<td>$&gt; .2$</td>
</tr>
<tr>
<td>Between Media</td>
<td>1</td>
<td>66.423</td>
<td>66.423</td>
<td>9.065</td>
<td>$&lt; .05$</td>
</tr>
<tr>
<td>Interaction</td>
<td>1</td>
<td>8.123</td>
<td>8.123</td>
<td>1.108</td>
<td>$&gt; .2$</td>
</tr>
<tr>
<td>Error</td>
<td>12</td>
<td>87.93</td>
<td>7.328</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Analysis of Variance of $t_{50}$

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>d.f.</th>
<th>S.S.</th>
<th>M.S.</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Preparations</td>
<td>1</td>
<td>7.156</td>
<td>7.156</td>
<td>1.815</td>
<td>$&gt; .2$</td>
</tr>
<tr>
<td>Between Media</td>
<td>1</td>
<td>26.266</td>
<td>26.266</td>
<td>6.661</td>
<td>$&lt; .05$</td>
</tr>
<tr>
<td>Interaction</td>
<td>1</td>
<td>20.026</td>
<td>20.026</td>
<td>5.079</td>
<td>$&lt; .05$</td>
</tr>
<tr>
<td>Error</td>
<td>12</td>
<td>47.318</td>
<td>3.943</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE III: The kinetics of lachesine when the preparation is bathed in a Krebs solution rather than in Tyrode's solution

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>t_{on}' (mins)</th>
<th>t_{off}' (mins)</th>
<th>(t_{off} - DR_{F+SL})/DR_{F+SL} (mins)</th>
<th>DR_{SL}</th>
<th>DR_{F+SL}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact pieces of ileum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>229 XI</td>
<td>4.5</td>
<td>29.7</td>
<td>7.0</td>
<td>12.0</td>
<td>44.9</td>
</tr>
<tr>
<td>230 I</td>
<td>3.5</td>
<td>21.7</td>
<td>8.2</td>
<td>14.3</td>
<td>38.8</td>
</tr>
<tr>
<td>231 II</td>
<td>3.7</td>
<td>10.8</td>
<td>3.7</td>
<td>10.1</td>
<td>33.9</td>
</tr>
<tr>
<td>232 II</td>
<td>2.9</td>
<td>11.7</td>
<td>4.7</td>
<td>14.1</td>
<td>47.7</td>
</tr>
<tr>
<td>Mean±S.E.M.</td>
<td>3.7±20.3</td>
<td>18.5±4.5</td>
<td>5.9±21.0</td>
<td>12.6±1.0</td>
<td>41.2±3.0</td>
</tr>
<tr>
<td>Longitudinal muscle strips</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>228 XI</td>
<td>2.2</td>
<td>18.2</td>
<td>5.3</td>
<td>16.5</td>
<td>48.8</td>
</tr>
<tr>
<td>229 I</td>
<td>3.5</td>
<td>29.7</td>
<td>5.0</td>
<td>15.3</td>
<td>37.7</td>
</tr>
<tr>
<td>230 II</td>
<td>2.3</td>
<td>16.1</td>
<td>4.1</td>
<td>13.8</td>
<td>43.8</td>
</tr>
<tr>
<td>232 II</td>
<td>3.0</td>
<td>28.1</td>
<td>4.2</td>
<td>14.9</td>
<td>41.0</td>
</tr>
<tr>
<td>Mean±S.E.M.</td>
<td>2.8±20.3</td>
<td>23.0±3.4</td>
<td>4.7±20.3</td>
<td>15.1±0.6</td>
<td>42.8±2.4</td>
</tr>
</tbody>
</table>

DR_{SL} is the equilibrium dose ratio produced by $2 \times 10^{-8}$M lachesine

t_{on}' and t_{off}' were calculated from the first three responses after the antagonist was added or removed.

[\textit{t_{off}}, the time constant for the rate of decrease of lachesine's occupancy on superimposing $15 \times 10^{-8}$M pentyl TEA, was calculated from the first three responses after the fast antagonist was added.]

Details

Preparation - intact pieces of ileum or muscle strips
Bathing solution - Krebs
Lever - isotonic
Antagonists - $2 \times 10^{-8}$M lachesine (SL)
- $15 \times 10^{-8}$M pentyl TEA (F)
Agonist - carbachol
### Analysis of Variance of $t_{off}$

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>d.f.</th>
<th>S.S.</th>
<th>M.S.</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Preparations</td>
<td>1</td>
<td>36.603</td>
<td>36.603</td>
<td>0.273</td>
<td>.05 P &lt; .1</td>
</tr>
<tr>
<td>Between Media</td>
<td>1</td>
<td>615.05</td>
<td>615.05</td>
<td>4.581</td>
<td>.05 P &lt; .1</td>
</tr>
<tr>
<td>Interaction</td>
<td>1</td>
<td>9.303</td>
<td>9.303</td>
<td>0.069</td>
<td>.001 P &lt; .01</td>
</tr>
<tr>
<td>Error</td>
<td>12</td>
<td>1611.195</td>
<td>134.266</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Analysis of Variance of $t_{off}^{DR_{T}+SL}/(DR_{T}+SL-DR_{SL}+1)$

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>d.f.</th>
<th>S.S.</th>
<th>M.S.</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Preparations</td>
<td>1</td>
<td>11.888</td>
<td>11.888</td>
<td>3.628</td>
<td>.05 P &lt; .1</td>
</tr>
<tr>
<td>Between Media</td>
<td>1</td>
<td>6.488</td>
<td>6.488</td>
<td>1.981</td>
<td>.1 P &lt; .2</td>
</tr>
<tr>
<td>Interaction</td>
<td>1</td>
<td>6.138</td>
<td>6.138</td>
<td>1.874</td>
<td>.1 P &lt; .2</td>
</tr>
<tr>
<td>Error</td>
<td>12</td>
<td>39.307</td>
<td>3.275</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

These comparisons therefore suggest that there are significant differences (P < .05), in $DR_{co}$ and $t_{on}$.

As shown in DIAGRAM III.18 the rates of onset and offset appear to be markedly faster when Krebs solution is used rather than Tyrode's, when longitudinal muscle strips are used. However, as shown in DIAGRAM III.17 the rates of onset and offset do not appear to be markedly faster when Krebs solution is used rather than Tyrode's, if intact pieces of ileum are used.
Diagram III.17: The kinetics of onset and offset of lachesine - comparing the use of Krebs solution with using Tyrode's (intact pieces of ileum).

For onset, mean values of \((P_{oo} - P_t)\) ± S.E.M. are plotted on a log scale against time.

For offset, mean values of \((P_t)\) ± S.E.M. are plotted on a log scale against time.

Details (See Tables III.9 and III.16)
Preparation - intact pieces of ileum
Bathing solution - Tyrode's
- Krebs  n=16  Mean DR_{oo} ± S.E.M. = 14.9 ± 1.0
- Tyrode's  n=16  Mean DR_{oo} ± S.E.M. = 12.6 ± 1.0

Lever - isotonic
Antagonist - \(2 \times 10^{-6}\)M lachesine
Agonist - carbachol
DIAGRAM III.18: The kinetics of onset and offset of lachesine - comparing the use of Krebs solution with the use of Tyrode's (muscle strips)

Details (see also TABLE III.9 & TABLE III.16)
- Bathing solution:
  - Tyrode's
  - Krebs
- Preparation: muscle strips
- Lever: isotonic
- Antagonist: 2 x 10^-8M lachesine
- Agonist: carbachol
Diagram III.19: The rate of offset of lachesine on superimposing pentyl TEA - comparing the use of Krebs solution with the use of Tyrode's solution
(a) using intact pieces of ileum  (b) using muscle strips
Mean values of \((p_C - p_{oo}) \pm S.E.M\) are plotted on a log scale against time

Details (see also TABLES III.9 and III.16)
Preparation - (a) intact pieces of ileum
- (b) muscle strips
Lever - isotonic
Bathing solution - Tyrode's - Krebs
Antagonists - \(2 \times 10^{-5}\) lachesine
- \(15 \times 10^{-6}\)M pentyl TEA
Agonist - carbachol
Discussion of Sections III.1-5

1. The discrepancies found in Parts I and II between the kinetics of lachesine and BTrMe and the predictions of the interaction limited model

In III.1-5 the kinetics of BTrMe and lachesine were followed using a variety of experimental methods; longitudinal muscle strips or intact pieces of ileum were used; an isometric transducer or an isotonic lever; pentyl TMA or carbachol, and Tyrode's solution or Krebs. In no case was the rate of recovery from antagonism fast enough to be limited by the rate of dissociation of the antagonist from the receptors; \( t_{\text{off}} \) was always much greater than the limiting value of \( \frac{t_{\text{FDR}}}{t_{\text{DR}}} \). It therefore seems unlikely that the discrepancies described in I and II between the kinetics of lachesine and BTrMe and the predictions of the interaction limited model are due to the method used.

2. The effect of the agonist, lever, preparation and bathing solution on the kinetics of antagonism

There was no reason to believe that the kinetics of antagonism would be affected by whether carbachol or pentyl TMA was used. In III.2 and 3, experiments in which carbachol was used to follow the kinetics of BTrMe and lachesine are compared with those in which pentyl TMA was used. The experiments indicate that the kinetics of antagonism was not markedly effected by whether carbachol or pentyl TMA was used.

These experiments did show that the equilibrium dose ratio produced by BTrMe tended to be slightly larger when pentyl TMA was used compared with when carbachol was used. This discrepancy was investigated further as described in the Appendix.

Comparisons are made in III.1 and III.3 between longitudinal
muscle strips and intact pieces of ileum and also between an isometric transducer and an isotonic lever. In III.1 experiments in which the kinetics of BTrMe was followed using muscle strips and an isometric transducer are compared with those in which intact pieces of ileum and an isotonic lever was used. When muscle strips were used rather than intact pieces of ileum, the rates of both onset and offset were slower, the relationship between log occupancy and time appeared to be more linear and the rate of offset did not appear to depend on the concentration of BTrMe used. In III.3 the kinetics of lachesine using intact pieces of ileum are compared with those when muscle strips were used. The rates of onset and offset do not appear to be markedly affected by whether intact pieces of ileum or muscle strips were used.

This suggests that the differences noted in III.1 might be due to the levers used rather than the preparations. However the comparisons made in III.4 between the kinetics of lachesine using intact pieces of ileum or muscle strips, and using an isotonic lever or isometric transducer, do not indicate that there were significant differences in the initial rates of onset and offset between groups, (P < .05). However comparisons of the overall rates as illustrated in the diagrams indicate that there may be small differences. Such differences might not be statistically significant in the analyses of variance of the initial rates due to the small number of observations in each group and the variances of the groups.

In addition the analyses of variance do not take into account the fact that two experiments were often done per animal and two sets of apparatus were used. Also although the order in which the experiments were performed was 'random' there was a restriction in that, for practical reasons the isometric experiments were paired. As the analysis of variance does not take these effects into account this may
account for the large number of variance ratios less than 1. If
these effects could be taken into account, this would make the
error sum of squares smaller and consequently the variance ratios
larger. The analysis might then indicate significant differences.

The values of $t_{off}$, $t_{off}^{DR_P<9L/DR_p}$ and DIAGRAM III.13 are
consistent with the suggestion, from the results of III.1 and III.3,
that the use of an isometric lever may slow the kinetics of antagonism
as compared with using an isotonic lever. If this is
the case, the effects might be due to changes in the geometry of the
tissue; Freeman-Harrod & Goodford (1962) found that if the tension in
the smooth muscle of guinea-pig taenia coli was increased, the rate of
potassium uptake was also increased. They found that this increase
could be quantitatively explained by the increased ratio of cell
surface to cell volume.

If the lever used does influence the kinetics of antagonism, it
is possible that the use of different levers could contribute to
discrepancies in kinetic observations between different groups of
workers. For instance in Parts I and II an isotonic lever was used
whereas Paton & Rang (1965) used an auxotonic lever. In addition
the load on the lever, (in the case of an isotonic or auxotonic lever,) or the tension which the tissue is under when no agonist is present,
(in the case of an isometric lever), might also contribute to the
variability of kinetic measurements.

Although these studies rule out the possibility that the kinetics
of antagonism is appreciably faster when muscle strips are used rather
than intact pieces of ileum, there is a tendency for the kinetics of
antagonism to be more variable and slower when muscle strips are used.
This may be associated with the tendency for the odd experiment to be
much slower than expected; throughout these studies the odd experiment occurred in which the rates of antagonism were much slower than would be expected if the observations were normally distributed. This tendency appeared to be more marked when muscle strips were used. Such variation may be connected with different amounts of damage done to the tissue in its preparation.

In the preparation of muscle strips and also intact pieces of ileum, the tissue is damaged to a greater or less extent. This could cause a variation in the amount of potassium lost, Goodford & Hermanssen (1961), and potassium depletion appears to slow the kinetics of antagonism, Paton (1967)b. Increased disturbance of the ionic balance of the tissue when muscle strips are prepared, as compared with the preparation of intact pieces of ileum, might account for the tendency for such preparations to give more variable and slower kinetic measurements.

In III.5 the kinetics of lachesine using tissues bathed in Krebs solution are compared with those in which the tissue was bathed in Tyrode's solution. If muscle strips are used, the rates of onset and offset appear to be markedly faster when the preparation is bathed in Krebs solution rather than Tyrode's. This differences is not so marked when intact pieces of ileum are used. This is consistent with Paton & Rothschild's (1965)a supposition that muscle strips would be more influenced by changes in the ionic composition of the bathing solution than would intact pieces of ileum.

Several previous observations have also connected the observed kinetics of antagonism with the ionic environment. For instance Beraldo & Rocha e Silva (1949) found that the kinetics of recovery of intact pieces of ileum from antihistamines and atropine were effected by the concentrations of calcium, magnesium and potassium, potassium and magnesium displaying strikingly antagonistic effects; decreasing
the concentrations of potassium or calcium below that of normal Tyrode decreased the rate of recovery, whereas increasing the concentrations of magnesium decreased the rate of recovery. They also noticed that the cations potassium and magnesium appeared to affect the course of recovery from inhibition by antihistamines and atropine in a very similar way.

Paton & Rothschild (1965)a also investigated the effect of calcium deficiency on the rates of onset and recovery of longitudinal muscle strips from guinea-pig ileum from hyoscine and mepyramine. They found that calcium deficiency reduced the rates of onset and offset and that this was not associated with an alteration in the equilibrium constant. Paton's observation (1967)b that potassium depletion slowed the rate of recovery from antagonism is also consistent with Beraldo's

As Krebs solution differs from Tyrode's solution in its ionic composition containing more potassium, (5.9 mM compared with 2.7 mM), and more calcium, (2.5 mM compared with 1.8 mM), a difference between using Krebs solution and Tyrode's could be due to their ionic composition. This does not mean though that variations in pH, tonicity or other factors might not also contribute.

In III.5 the use of Krebs solution, as against Tyrode's, did appear to decrease the dose ratio produced by lachesine. However it seems unlikely that the differences in kinetic behaviour could be due to this because Paton & Rothschild (1965)a observed that the calcium ion concentration appeared to influence the kinetics of antagonism without altering the equilibrium constant. They interpreted their observations in terms of a calcium-binding site associated with the receptor area which facilitates receptor reactivity. This explanation seems unlikely in view of the evidence that the kinetics of antagonism appear to be access limited in such a situation. In addition the
rate of offset of lachesine on superimposition of pentyl TEA was not found in 1.5 to be markedly effected by the bathing solution.

Why varying the bathing media should influence the kinetics of antagonism, if they are access limited, is not clear. It might be related to induced changes in the geometry of the tissue. This could increase or decrease the diffusion distance between the bulk of the bathing solution and the receptors or, in terms of Rang's limited biophase model, the volume of the biophase could be altered. Goodford & Leach (1966) found that if isolated guinea-pig taenia coli were left in contact with Krebs solution for 3 hours the cells shrank, increasing the inulin space but not the sucrose space. In addition various ions have been found to be involved in maintaining cell volume. For instance, Brading & Tomita (1968) found that if frog stomach muscles were placed in a Locke's solution in which the sodium chloride was replaced by sucrose, the tissue shrank by 20% if 2.2 mM calcium was present but swelled by 20% in the absence of calcium.

Similarly Bozler (1962) compared the swelling of frog stomach muscle when placed in water or dilute solutions of magnesium or calcium chloride, or solutions of sodium chloride. In the magnesium or calcium chloride the cells swelled by about 15-30% in one hour compared with about five times this amount in water. He concluded though that the difference was due to the presence of calcium or magnesium ions rather than osmotic pressure because in a sodium chloride solution of the same osmotic pressure the gain in weight was as rapid as if they had been put in just water.

In addition, although variation in the ionic composition of the extracellular space would not be expected to alter appreciably the diffusion velocity of antagonists through this space, if the access limitation involved a barrier to diffusion such as the basement
membrane, the ionic environment might alter the rate at which antagonists diffuse through this layer.

The differences between the kinetic observations made by different groups of workers may be associated, in certain circumstances, with the use of different bathing media. For instance in Part II Tyrode's solution was used whereas Paton & Rang (1965) used a Krebs solution; in this case the discrepancy between the two studies may be contributed to by the difference in bathing media. However discrepancies have also been observed between investigations in which the same media has been used. For instance Paton (1961) and Paton & Rothschild (1965) both used a Krebs-Henseleit solution and therefore discrepancies between these investigations are unlikely to have been caused by differences in the ionic composition of the bathing medium.

The Krebs-Henseleit solutions used were however supplemented by varying amounts of glucose or dextrose: Paton (1961) used 1 g/l glucose, Paton & Rang (1965) used 2.1 g/l dextrose and Waud (1969) used 20.8 g/l glucose. As Burgen (1966) pointed out, smaller molecules such as sucrose are more effective in modifying the diffusion velocity of other molecules than their effect on viscosity might suggest. Therefore varying amounts of glucose and dextrose might effect the diffusion velocity of antagonistic drugs.
3. The discrepancies between the kinetic observations made by different groups of workers

The rate of recovery from atropine's antagonism has been followed by several different groups of workers and the rates found differ markedly. Initially Paton (1961) using intact pieces of ileum found that atropine washed out with a time constant of about 56 minutes. Then Paton & Rang (1965) using longitudinal muscle strips found that atropine washed out with a time constant of about 10 minutes.

It therefore seemed reasonable to suggest, e.g. Paton (1967)a, that the thinness of muscle strips compared to intact pieces of ileum decreased delays due to diffusion through the tissue and this increased the observed rates of antagonism. However this explanation seems inadequate because:

1. Subsequent observations of atropine's action do not confirm the original correlation; Furchgott, (quoted by Paton (1967)b), using intact pieces of guinea pig ileum measured offset half times nearer ten minutes than 40; conversely, Thron & Waud (1968) using muscle strips found that atropine washed out with a time constant much slower than 10 minutes. (They do not quote a value for the time constant but the slowness is shown in their Fig. 8.)

2. Comparisons between the two preparations using other antagonists do not show the same differences. For instance Paton (1961) using intact pieces of ileum observed that the time constant for offset of hyoscine's action was 64 minutes whereas Paton & Rothschild (1965) using longitudinal muscle strips found that hyoscine washed out with a time constant of 64 minutes.

3. In addition in III.4 no evidence was found that the kinetics of antagonism was faster when muscle strips were used rather than intact pieces of ileum.
If atropine was the only antagonist whose kinetics varied considerably from worker to worker one could suggest that the differences were associated with different degrees of uptake into the cells due to variations in the pH of the bathing medium. This might also explain why the rate of recovery from atropine gets slower as the concentration of atropine is increased.

However large discrepancies have also been found using quaternary compounds such as lachesine and for these compounds intracellular uptake would be expected to be less marked. Therefore although intracellular uptake and pH considerations may contribute to the variation in atropine's case it is likely that other factors or combinations of factors are involved.

In addition to the possible influence of the preparation type, lever and bathing medium already discussed, the actual size of the animal could influence the kinetics of antagonism, perhaps due to variation in the geometry of the tissue with age. The basement membrane of certain cells has been found to increase with age, Pierce, Beals, Ram and Midgley (1964), and changes such as this might influence the observed kinetics of antagonists. Alternatively the amount of damage done to the tissue in preparation might also vary with the size of the animal from which the preparation is taken. This could therefore contribute to differences between experiments in which different sized animals are used. For instance in Part III care was taken that the animals from which strip preparations were taken were of the same weight range as those used for the other preparation, (150-400g), whereas Paton & Rang (1965) specify that they used animals over 500g in weight.

Another possibility is that the different rates of recovery are due to different concentrations of antagonist used. Unfortunately
in almost every case the investigators do not state what range of concentrations of antagonist were investigated and so it is impossible to eliminate this possibility. This effect would be particularly important if the rate of offset was not independent of the concentration of the concentration of antagonist used.

Although there seems to be no single explanation to account for all the discrepancies, the factors which influence the kinetics of antagonism are of considerable interest because they provide insight into the nature of the access limitation.
126

III.6: THE RELATIONSHIP BETWEEN THE LIMITING VALUE OF THE RATE OF OFFSET AND $k_2$ THE ANTAGONIST-RECEPTOR DISSOCIATION RATE CONSTANT

The limiting value of the rate of offset of an antagonist on superimposition of high concentrations of a fast antagonist may be dissociation limited. If this is the case values of $k_2$ could be determined from it and the values obtained would be expected to be independent of many experimental variables.

In III.5 the lever, preparation and bathing medium used did not appear to significantly affect, ($P < .05$), the value of $t_{off}^{DR_{P_{TEA}}}/DR_{P_{TEA}}$. However the rates were slower when an isometric transducer was used rather than an isotonic lever. Therefore the rate of offset of lachesine on superimposition of $25 \times 10^{-4}M$ pentyl TEA was investigated using an isometric transducer, together with intact pieces of ileum, to determine whether this increased the rate of offset as compared with the experiments in which $15 \times 10^{-4}M$ was used.

In addition experiments were carried out to determine whether there was any evidence that the limiting value was influenced by the particular fast antagonist. The rate of offset of lachesine on superimposition of octyl TMA was examined because octyl TMA has the same molecular weight as pentyl TEA and would therefore be expected to have similar diffusion properties. The offset of lachesine on superimposition of diphenylacetoxyethyldimethylethyl ammonium, $(Ph_2AOEMe_2Et)$ was also examined as initial experiments had shown that it acted just as fast as pentyl TEA and its rate of offset did not appear to change as its concentration was increased.

Procedure

The rate of offset of $2 \times 10^{-8}M$ lachesine on superimposition of
25 x 10^{-4}M pentyl TEA was followed as in II.2 except that an isometric transducer was used to record the responses.

The rates of offset of 2 x 10^{-8}M lachesine on superimposition of 8.9 and 15 x 10^{-6}M octyl TMA, or 40 x 10^{-7}M Ph_2A0EMe_2Et were followed as in II.2.

Results

These experiments are illustrated in DIAGRAMS III.21, 24, 25.

Values of t_{off} were calculated for the individual experiments from the first three responses after the fast antagonist was added. These values are indicated in TABLES III.20 and III.22.

The values of t_{off}^{DR_+SL/DR_{-}} were examined by means of a variance analysis together with the results considered in III.4 in which intact pieces of ileum were used, i.e. 6 groups with 4 observations in each.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>d.f.</th>
<th>S.S.</th>
<th>M.S.</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>5</td>
<td>114.94</td>
<td>22.988</td>
<td>2.790</td>
<td>.05 &gt; P &gt; .01</td>
</tr>
<tr>
<td>Error</td>
<td>18</td>
<td>148.3</td>
<td>8.239</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This indicates that there are significant differences (P < .05) between the groups.

Paired t tests between groups indicated that when an isometric lever was used, 25 x 10^{-4}M pentyl TEA gave significantly lower values of t_{off}^{DR_+SL/DR_{-}} than when 15 x 10^{-4}M was used (P < .05); 25 x 10^{-4}M pentyl TEA (isometric transducer) did not give significantly lower values than when 15 x 10^{-4}M pentyl TEA was used, (isotonic lever). The following comparisons were also made and were not found to be significant:

15 x 10^{-4}M octyl TMA was compared with 15 x 10^{-4}M pentyl TMA; 15 x 10^{-4}M
TABLE III.20 & DIAGRAM III.21: The rate of offset of $2 \times 10^{-8}$M lachésine on superimposing $25 \times 10^{-4}$M pentyl TEA.

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>$t_{\text{off}}$ (DR&lt;sub&gt;F+SL&lt;/sub&gt; / DR&lt;sub&gt;P&lt;/sub&gt; mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>234 I</td>
<td>2.9</td>
</tr>
<tr>
<td>235 I</td>
<td>3.0</td>
</tr>
<tr>
<td>236 I</td>
<td>4.0</td>
</tr>
<tr>
<td>237 I</td>
<td>1.4</td>
</tr>
<tr>
<td>Mean ± S.E.M.</td>
<td>2.8 ± 0.5</td>
</tr>
</tbody>
</table>

Mean values of $(p_t - p_{oo})/S.E.M.$ are plotted on a log scale against time.

Details: (see also TABLE III.9)
Preparation - intact pieces of ileum
Bathing solution - Tyrode's
Lever - isometric
Antagonists - $2 \times 10^{-8}$M lachésine
- (a) $15 \times 10^{-4}$M pentyl TEA
- (b) $25 \times 10^{-4}$M pentyl TEA
Agonist - carbachol

----- corresponds to a time constant $t_{\text{off}} = 4 \times \text{mean DR}_P \text{ mins}$

$\text{mean DR}_F+\text{SL}$
### TABLE III.22: The rate of offset of lachesine on superimposing octyl TMA or Ph₂AOEM₂Et

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Fast Antagonist</th>
<th>Concentration of Fast Antagonist (M)</th>
<th>$t_{off}$ (mins)</th>
<th>DRₐ₊SL (\overline{DR}_{L} \pm S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>219 I</td>
<td>Octyl TMA</td>
<td>$8.9 \times 10^{-4}$</td>
<td>5.3</td>
<td></td>
</tr>
<tr>
<td>222 II</td>
<td>Octyl TMA</td>
<td>$8.9 \times 10^{-4}$</td>
<td>16.0</td>
<td></td>
</tr>
<tr>
<td>223 I</td>
<td>Octyl TMA</td>
<td>$8.9 \times 10^{-4}$</td>
<td>6.6</td>
<td></td>
</tr>
<tr>
<td>227 I</td>
<td>Octyl TMA</td>
<td>$8.9 \times 10^{-4}$</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>Mean ± S.E.M.</td>
<td></td>
<td></td>
<td>8.1 ± 2.7</td>
<td></td>
</tr>
<tr>
<td>234 II</td>
<td>Octyl TMA</td>
<td>$15 \times 10^{-4}$</td>
<td>7.9</td>
<td></td>
</tr>
<tr>
<td>235 I</td>
<td>Octyl TMA</td>
<td>$15 \times 10^{-4}$</td>
<td>5.9</td>
<td></td>
</tr>
<tr>
<td>236 I</td>
<td>Octyl TMA</td>
<td>$15 \times 10^{-4}$</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>237 I</td>
<td>Octyl TMA</td>
<td>$15 \times 10^{-4}$</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Mean ± S.E.M.</td>
<td></td>
<td></td>
<td>4.3 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>237 I</td>
<td>Ph₂AOEM₂Et</td>
<td>$40 \times 10^{-7}$</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>234 I</td>
<td>Ph₂AOEM₂Et</td>
<td>$40 \times 10^{-7}$</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>235 I</td>
<td>Ph₂AOEM₂Et</td>
<td>$40 \times 10^{-7}$</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>236 II</td>
<td>Ph₂AOEM₂Et</td>
<td>$40 \times 10^{-7}$</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>Mean ± S.E.M.</td>
<td></td>
<td></td>
<td>2.6 ± 0.5</td>
<td></td>
</tr>
</tbody>
</table>

**Details**
(see also TABLE III.23)

Preparation - intact pieces of ileum
Bathing solution - Tyrode’s
Lever - isotonic
Antagonists - 2 x $10^{-8}$M lachesine
- $8.9 \times 10^{-4}$M octyl TMA
- $15 \times 10^{-4}$M octyl TMA
- $40 \times 10^{-7}$M Ph₂AOEM₂Et

Agonist - carbachol
**TABLE III**. The interaction between $2 \times 10^{-6}$M lachesine and, octyl TMA or Ph$_2$ACEm$_2$Et: comparing the observed dose ratios $A_{SL+F}$/$A_{SL}$ with that calculated assuming the antagonists to be competitive.

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Fast Antagonist</th>
<th>Concentration of Fast Antagonist M</th>
<th>Observed Dose Ratio $A_{SL+F}$/$A_{SL}$</th>
<th>Calculated Dose Ratio $\frac{SL K_{SL+D} F K_{P+1}}{SL K_{SL+1}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>219 I</td>
<td>Octyl TMA</td>
<td>$8.9 \times 10^{-4}$</td>
<td>4.6</td>
<td>4.9</td>
</tr>
<tr>
<td>222 II</td>
<td>Octyl TMA</td>
<td>$15 \times 10^{-4}$</td>
<td>8.7</td>
<td>7.6</td>
</tr>
<tr>
<td>223 I</td>
<td>Octyl TMA</td>
<td>$2.9 \times 10^{-4}$</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>227 I</td>
<td>Octyl TMA</td>
<td>Mean ± S.E.M.</td>
<td>$3.5 \pm 0.6$</td>
<td></td>
</tr>
<tr>
<td>234 II</td>
<td>Octyl TMA</td>
<td>$40 \times 10^{-7}$</td>
<td>12.3</td>
<td></td>
</tr>
<tr>
<td>235 I</td>
<td>Ph$_2$ACEm$_2$Et</td>
<td>$9.0 \times 10^{-7}$</td>
<td>9.0</td>
<td></td>
</tr>
<tr>
<td>236 I</td>
<td>Ph$_2$ACEm$_2$Et</td>
<td>Mean ± S.E.M.</td>
<td>$10.5 \pm 0.9$</td>
<td></td>
</tr>
</tbody>
</table>

$A_{SL+F}/A_{SL}$ is the ratio of the concentration of agonist required at equilibrium in the presence of the slow and the fast antagonist, to produce the same response as $A_{SL}$ in the presence of the slow only, to $A_{SL}$.

If the two antagonists are competitive this ratio will be equal to $(SL K_{SL+D} F K_{P+1})/(SL K_{SL+1})$. This was calculated using the following values for the affinity constants:

- **lachesine** $K_{SL} = 6.6 \times 10^6$M$^{-1}$ (in 26 estimations mean log $K_{SL} S.E.M. = 8.817 \pm 0.027$)
- **octyl TMA** $K_{P} = 6.3 \times 10^4$M$^{-1}$ (the mean value determined by Stephenson, 1956)
- **Ph$_2$ACEm$_2$Et** $K_{P} = 3.9 \times 10^7$M$^{-1}$ (in 16 estimations mean log $K_{P} S.E.M. = 7.396 \pm 0.029$)

**Details**
- **Preparation** - intact pieces of ileum
- **Bathing solution** - Tyrode's
- **Lever** - isotonic
- **Agonist** - carbachol
Diagram III.24: The rate of offset of lachesine on superimposing octyl TMA

Mean values of $(p_c - p_{co})\pm$ S.E.M. are plotted on a log scale against time

Details (see also Table III.22)

Preparation - intact pieces of ileum

Bathing solution - Tyrode's

Lever - isotonic

Antagonists - $2 \times 10^{-8}M$ lachesine
- octyl TMA (a) $8.9 \times 10^{-4}M$ (b) $15 \times 10^{-4}M$

Agonist - carbachol

----- corresponds to a time constant $t_{off} = 4 \times DR_{p}/DR_{p+SL}$ mins
DIAGRAM III.25: The rate of offset of lachesine on superimposing \( \text{Ph}_2\text{AOEt}_2\text{Et} \)

Mean values of \( (P_t - P_{oo}) \pm \text{S.E.M.} \) are plotted on a log scale against time

Details
(see also TABLE III.22)
Preparation - intact pieces of ileum
Bathing solution - Tyrode's
Lever - isotonic
Antagonists - \( 2 \times 10^{-8} \text{M} \) lachesine
\( 40 \times 10^{-7} \text{M} \text{Ph}_2\text{AOEt}_2\text{Et} \)
Agonist - carbachol

\[ \text{corresponds to a time constant } t_{off} = \frac{4 \times \text{mean D}_{p_{oo}} \pm \text{mean D}_{p_{oo} + SL}}{\text{mins}} \]
octyl TMA was compared with $8.9 \times 10^{-6}$M octyl TMA; and $40 \times 10^{-7}$M Ph$_2$AOEMe$_2$Et was compared with $15 \times 10^{-4}$M pentyl TMA (isotonic lever).

As in I.2 and II.2, the calculations of lachesine's occupancy assumes that it competes with the fast antagonist being used; TABLE III.23 compares the observed dose ratio $A_{SL}/A_{FS}$ with that calculated assuming the antagonists to be competitive. This shows that the fast antagonists combine with lachesine as expected for two competitive antagonists despite the fact that octyl TMA is normally considered as a partial agonist.

The calculations also assume that the difference between the rates of the fast and slow antagonist is sufficiently large that:

$$P_F = \frac{F K_F (1 - P_{SL})}{1 + F K_F}$$

The rates of onset and offset of octyl TMA could not be determined because it is a partial agonist. However Paton (1961) estimated $t_{off}$ to be less than 20 seconds from the rate of its antagonistic action on coaxially stimulated pieces of guinea-pig ileum. This is in the same order as that found using equivalent concentrations of pentyl TZA.

Also no quantitative measurements could be made of the kinetics of Ph$_2$AOEMe$_2$Et because its recovery was frequently associated with a transient increase in the resting tension of the tissue. In addition determinations of $P_{SL}$ would be complicated by the fact that in every experiment there appeared to be a slight increase in the slope of the log dose response relationship. (This was not apparent in those experiments in which lachesine was present throughout.)
Discussion

When the rate of offset of lachesine on superimposition of pentyl TEA was followed under different experimental conditions, although the rate of offset varied under the different conditions, there was no evidence that the limiting value of $t_{\text{off}}^{\text{DR}_{F+SL}}/\text{DR}_F$ was markedly affected. The same applies to the rate of offset on superimposition of octyl TMA or $\text{Ph}_2\text{AOEMe}_2\text{Et}$. This is consistent with the limiting value of $t_{\text{off}}^{\text{DR}_{F+SL}/\text{DR}_F}$ being equal to $1/k_2$, $k_2$ being the antagonist-receptor dissociation rate constant. However it is possible that a different access limitation is operating. This can not be ruled out particularly as so little is known about the access of a drug to the receptors.

In addition, as the number of observations in each group was small, there might be a small difference in limiting-value which would not be 'significant' in such groups. In particular the rate of offset on superimposition of $\text{Ph}_2\text{AOEMe}_2\text{Et}$ does appear to be faster than the limiting value indicated in DIAGRAM II.7, but as this concentration produces a larger dose ratio than that produced by $15 \times 10^{-4}\text{M}$ pentyl TEA it may just be nearer the 'true' limiting value.
III.7 THE KINETICS OF BTMMe FOLLOWING TREATMENT OF THE TISSUE WITH AN IRREVERSIBLE ANTAGONIST

Thron & Waud (1968) studied the effect of incubating longitudinal muscle strips with dibenamine on the subsequent rates of onset and offset of atropine's action. They found that the rates of both onset and offset were accelerated, although the equilibrium constant was apparently unchanged. This observation is important because it supports any access-limited model in which the rate at which the concentration of a drug in the proximity of the receptors changes is slowed by receptor uptake, i.e. any model of the limited type such as that of Rang (1966). The effect of pretreating intact pieces of ileum with the irreversible antagonist SY 19 on the kinetics of BTMMe was therefore investigated to see if Thron & Waud's original observation could be substantiated.

Procedure

The SY 19 used was initially dissolved in acid-alcohol, this solution being subsequently diluted in Tyrode's solution before addition to the organ bath.

The tissue was set up as in I.1 and allowed to settle down for a period of about an hour, responses being produced every 90 seconds. 5 ug of SY 19 was then pipetted into the organ bath and left in contact with the tissue for about 10 minutes. It was then washed out. This was repeated until a block (after the fast initial recovery) equivalent to a dose ratio of ten was produced. After the initial block, 8 ug/litre of SY 19 was added to the wash and agonist solutions to try to maintain the block.

The onset and offset of $2 \times 10^{-10} M$ BTMMe was then followed in the usual way, i.e. a modification of the method of Paton & Rang (1965), as described in I.1.
In spite of the background presence of SY 19, control experiments showed that there was an appreciable recovery during the time required to watch the onset and offset of BT\textsubscript{Me}'s action. To allow for this in the calculations of BT\textsubscript{Me}'s occupancy, it was assumed that the equilibrium dose ratio due to the BT\textsubscript{Me} in the presence of SY 19 was what it would be expected to be from the Schild equation, i.e., \( \text{DR}_\infty = 1 + C.K_C \), \( C \) being the concentration of BT\textsubscript{Me} and \( K_C \) being the affinity constant of BT\textsubscript{Me}. The rate of recovery from the 'irreversible' block was then assumed to be constant from the moment the BT\textsubscript{Me} was added to when an equilibrium was established, and subsequently when the BT\textsubscript{Me} was removed. BT\textsubscript{Me}'s occupancy corresponding to each response was then calculated on this basis.

Results

The rates of onset and offset of BT\textsubscript{Me} after pretreatment with SY 19 are compared with those obtained in I.1, where the preparations had not been so treated, in DIAGRAM III.26. The rate of onset appears to have been accelerated by pre-treatment with SY 19 and there appears to be a 'more-linear' relationship between log occupancy and time. Although there was no appreciable acceleration in the rate of offset, this might well have been due to the decreased receptor block by the irreversible antagonist.

These results are consistent with Thron & Waud's (1968) observation and therefore support any access model of the limited type.
Diagram III.26 The kinetics of onset and offset of BTrMe - comparing the rates observed when the tissue had been 'pretreated' with SY 19, with the rates using 'untreated' tissues.

For onset, mean values of $(p_{oo} - p_c) \pm S.E.M.$ are plotted on a log scale against time.

For offset, mean values of $(p_c) \pm S.E.M.$ are plotted on a log scale against time.

Details:
- Preparation: intact pieces of ileum
- Bathing solution: Tyrode's
- Lever: isotonic
- Antagonist: BTrMe $20 \times 10^{-10} M$
- Agonist: carbachol

After treatment with SY 19
- n = 5 Exp. Nos. 197 I, 199 I, 199 II, 198 II, 201 II

Not treated with SY 19
- n = 4 Exp. Nos. 168 I, 167 II, 168 II, 169 I
III.8: THE KINETICS OF LACHESINE USING HEXYL TMA

Thron & Waud (1968) suggested that, if access was rate limiting, the large concentration gradient resulting when a slow antagonist is 'displaced' from the receptors by a partial agonist might accelerate the offset of the slow antagonist. The kinetics of onset and offset of $2 \times 10^{-8}$M lachesine was therefore followed using the agonist hexyl TMA instead of carbachol. Hexyl TMA is on the borderline between full agonists and partial agonists but is normally able to produce a maximum response in the absence of antagonist.

Procedure & Results

The rate of onset and offset of $2 \times 10^{-8}$M lachesine was followed as described in II.4 but hexyl TMA was used instead of carbachol.

In DIAGRAM III.27 these experiments are compared with the equivalent experiments in which carbachol was used. The rate of onset appears to be faster when hexyl TMA is used rather than carbachol, whereas the rate of offset does not appear to be effected.

The experiments in which hexyl TMA was used were complicated by the fact that the slope of the log dose response relationship when the tissue was in equilibrium with the lachesine was less than that before the antagonist was added. The equilibrium dose ratio, $DR_{SL}$, under these conditions was calculated as the concentration of hexyl TMA required in the presence of the antagonist to produce the same response as $4 \times 10^{-6}$M hexyl TMA in the absence of antagonist, to $4 \times 10^{-6}$M. The higher values of $DR_{SL}$ obtained using hexyl TMA, indicated on DIAGRAM III.27 are thus probably partly due to non-equilibrium effects. Another possible contributing effect is discussed in the Appendix to this study. (In all other experiments a parallel shift in the log dose
DIAGRAM III.27: The kinetics of onset and offset of $2 \times 10^{-6}$M lachesine - comparing the use of hexyl TMA with the use of carbachol

For onset, mean values of $(p_{oo} - p_t) \pm$ S.E.M. are plotted on a log scale against time.

For offset, mean values of $(p_t) \pm$ S.E.M. are plotted on a log scale against time.

Details (see also TABLE III.9)

- Bathing solution: Tyrode's
- Lever: isotonic
- Agonists: carbachol
- Agonists: hexyl TMA
- Preparation: intact pieces of ileum
- Antagonist: $2 \times 10^{-6}$M lachesine

- Mean $D_{Roo}$ S.E.M. = 14.9 ± 1.0
- Mean $D_{Roo}$ S.E.M. = 37.9 ± 3.7
response relationship was assumed as there was no evidence of non-equilibrium effects.)

Discussion

Because of the change in slope of the log dose response relationship, the interpretation of these experiments is difficult. However there is no evidence that the apparent rate of offset is increased when hexyl TMA is used rather than carbachol, despite the occurrence of non-equilibrium effects.
DISCUSSION

Certain observations concerning the kinetics of BTrMe, lachesine and pentyl TEA provide insight into the type of access limitation; several observations indicate that receptor binding must be taken into account.

In addition any acceptable access limited model must also be consistent with the following observations:

1. The kinetics of antagonists do not appear to be faster when longitudinal muscle strips are used instead of intact pieces of ileum.
2. As the concentration of BTrMe is increased as in I.1, the time constant for recovery gets smaller, whereas when the concentration of pentyl TEA is increased as in I.4, the time constant for recovery gets larger.
3. The relationship between the structure of a drug, its affinity and the observed rates of onset and offset of antagonism must also be considered.

These factors will now be considered.

Receptor Binding

As pointed out by Thron (1972) any linear access limited model predicts that if, for the onset of antagonism \( \frac{(DR_{oo} - DR_c)}{(DR_{oo} - 1)} \), and for the offset \( \frac{(DR_c - 1)}{(DR_{oo} - 1)} \), plotted against time, the curves should be superimposable. The kinetics of BTrMe (I.1) and lachesine (II.1) were examined in this way and the curves for onset and offset were not superimposable, neither with themselves nor with each other. The kinetics of these antagonists are therefore not linear and as discussed in the introduction the most likely cause for this would be the degree of binding to the receptors.
The following observations also suggest that the degree of binding to the receptors influences the kinetics of antagonists:

1. The apparent rate of offset of lachesine and BTrMe is accelerated by superimposition of a high concentration of a fast antagonist, (I.2 and II.2).

2. Pre-treatment of a tissue with an irreversible antagonist appears to accelerate the kinetics of antagonism, (III.7).

The kinetics of antagonism on longitudinal muscle strips or intact pieces of ileum

Thron & Waud (1968) suggested that if a drug is considered to travel by diffusion from the bathing medium through the extracellular space to the receptors, the most appropriate access-limited model would be based essentially on the Fick-equation with a term to represent binding of the drug. However such a system would also be described by a multicompartamental model in which the compartments are arranged in series, each containing receptors and having the same $N K_{\text{eff}}/V$. The unidirectional rate of outward diffusion of a substance from any compartment would be proportional to its concentration in that compartment and this would be related to $D$, the diffusion coefficient of the drug in the extracellular space.

Such a model would not explain why using longitudinal muscle strips does not increase the rates of antagonism as compared with using intact pieces of ileum: if diffusion into muscle strips approximates to diffusion from two sides into a plane sheet and diffusion into intact pieces of ileum approximates to diffusion from one side into a plane sheet, the rate of rise of concentration inside the strips would be expected to be faster than in the latter case. However the rates of antagonism are not faster when muscle strips were used.
In addition Rang (1966) considered, with the aid of an analogue computer, the case of two compartments arranged in series each containing receptors and having the same value of $MK_{\text{aff}}/V_0$. When he compared this model with that of the unicompartamental limited biophase model he found that it was less satisfactory in simulating the experimental results because the rate of change in occupancy deviated more markedly from the exponential and also the ratio of the rate constants for onset and decline of antagonism at different drug concentrations differed from that found experimentally.

A unicompartamental model would be more appropriate than a multicompartamental model if, for instance, an antagonist equilibrates relatively quickly with the bulk of the extracellular space, the rate of action of the drug being principally determined by the rate at which it travels from the bulk of the extracellular space to the receptors. Such a situation might therefore explain Rang's (1966) observations and also why the kinetics of antagonists are not faster when muscle strips are used as against intact pieces of ileum.

Such a situation could arise either if there was a diffusion barrier between the receptors and the bulk of the extracellular space or if there was a layer around the receptors in which diffusion was slower than that in the bulk of the extracellular space. Such a proposition is given credibility by the existence of the basement membrane which could provide the physical basis for such a system.

The observations of Krenjević & Mitchell (1960), (see also P.45), suggest that although acetylcholine diffuses through the bulk of the extracellular space of rat diaphragms at a rate similar to that in dilute aqueous solutions, there is also a slow acetylcholine fraction. If extracellular, this fraction might indicate that there is a layer around the cells from which acetylcholine diffuses slowly.
In addition the existence of a 'high resistance' layer has also been considered in connection with the diffusion of ions. For instance Frankenhaeuser & Hodgkin (1956) investigated the positive phase following spikes of isolated squid axons and found that this phase appeared to be due to an accumulation of potassium ions outside the membrane associated with the membrane's increased permeability to potassium during the second half of the spike. However this positive phase declined at a rate slower than that expected if the potassium ions were able to diffuse freely away from the membrane. They also obtained independent evidence for a high resistance layer around the squid axon from the discrepancy between the values of axoplasm resistivity obtained at high frequencies with transverse electrodes and those obtained with direct or alternating current flowing parallel to the nerve fibres.

Subsequently Greengard & Straub (1958) also came to a similar conclusion when they investigated the after potentials in mammalian non-myelinated nerve fibres which also appear to be due to an accumulation of potassium ions around the excitable membrane. They calculated that the sheath around the axons would provide less than 1/200 of the barrier to diffusion required to account for the rate of decline of the negative after potential, if the gap in the Schwann cell, seen in electron micrographs, was an aqueous phase. They therefore concluded that the diffusion of potassium must be restricted by some other barrier to diffusion perhaps associated with the basement membrane.

Further support for the existence of such a 'high resistance' layer comes from Niedergerke's (1956) investigations of the action of calcium and potassium on excised frog's ventricle stimulated periodically. He found that increasing the calcium concentration, or decreasing the potassium concentration, facilitated the contractions without increasing the size or duration of the action potential.
However the time course of the action of potassium, and probably also calcium, was too slow to be accounted for by diffusion through the extracellular space at a rate similar to that expected for dilute aqueous solutions, but too fast to involve equilibration with the intracellular electrolyte content. In addition he suggested that if ions were not able to diffuse freely away from the cells, the calcium loss from heart cells during exercise might increase the local concentration of calcium around the cells sufficiently to account for the 'staircase' phenomenon. This phenomenon is similar to that produced by increasing the concentration of calcium, and it occurs when a ventricle is stimulated periodically after a period of quiescence.

Positive and negative after effects are also observed following contractions of pieces of guinea pig ileum, as shown by the effect of a single response on the size of a subsequent response. Suppression, i.e. a negative effect, has frequently been observed following large responses, e.g. Cantoni & Eastman (1946), and in this case is not specific in that doses of acetylcholine suppress subsequent responses of acetylcholine or histamine and vice versa. In addition a potentiation, i.e. a positive effect, can also be observed as shown by Beraldo & Rocha e Silva (1949). In DIAGRAM III.28 the interaction between these after effects is shown, both following large, but not maximal, responses and also following small responses.

These after effects may be due to disturbances in the ionic environment around the cells. For instance, Paton (1961), observed that there was a close resemblance between the insensitivity of smooth muscle due to previous exposure to high doses of a stimulant drug and exposure to potassium deficient solutions. Subsequently Paton & Rothschild (1965) found that desensitization produced by previous exposure to high doses of acetylcholine is reduced by calcium deficiency and appears to be related, not to the changes in calcium or
Diagram XIII. 26: The influence of previous responses on the size of response produced by a concentration of carbachol

Response produced by concentration of carbachol

Carbachol concentrations (m) are indicated above responses.
potassium content, but to the gain in sodium.

In addition the time course of these after effects appear to be influenced by the concentration of ions in the bathing solution in a similar way to the kinetics of antagonism; Beraldo & Rocha e Silva (1949), found that the recovery of response height following a large response was influenced by the concentration of ions in a similar way to the recovery from antagonism. It was also noticed in the experiments with BTrMe and lachesine that there was a tendency for after effects to be more pronounced in those experiments in which the kinetics of antagonists were also slower than normal.

It is therefore tempting to speculate that a local accumulation of ions may contribute to the after effects observed following contractions of pieces of guinea-pig ileum and that the time course of such effects may also be partly determined by their rate of diffusion from a 'high-resistance' layer around the cells. Regardless of whether this is the case or not, the other studies do suggest that there is a layer around the cells from which ions diffuse slower than expected if the layer was a dilute aqueous solution, and if the diffusion of ions is retarded that of drug molecules might be similarly effected.

There is however another possibility that could account for the apparent equivalence between using muscle strips and using intact pieces of ileum. This possibility is that responses are produced in isolated pieces of tissue by the agonist acting on the peripheral cells, the excitation produced in these cells being conducted from cell to cell inwards towards the centre of the tissue, i.e. the agonist might act by setting up a pacemaker in the superficial cells.

Although there is no direct evidence as to whether agonists act in this way on the longitudinal muscle of guinea-pig ileum, there are two observations which suggest that such a mechanism would be
possible; the cells do have pacemaker activity in that spontaneous discharges are preceded by a phase of slow depolarization of the membrane, Bullbring (1957), and also they are able to conduct excitation from cell to cell, Bullbring, Burnstock & Holman (1958). In addition Cuthbert & Dunant’s (1970) study of the kinetics of agonists would seem to support such a mechanism.

They compared the rate of action of agonists on guinea-pig ileum with the predictions of an access-limited model in which it was assumed that the drug molecules travel through a layer of thickness \( l \) between the bulk of the bathing fluid and the receptors. They found that their model satisfactorily described the kinetics of agonists. In addition they calculated diffusion half times, the time required for the substance to reach half of the final concentration at the receptors, and from these they determined \( l \), assuming the diffusion coefficient of the drug to be constant and equal to that expected in dilute aqueous solutions. These values of \( l \) agreed with the size of the unstirred layer of liquid, called a stationary layer, found between a solid and a well stirred liquid in physico-chemical systems and also at the surface of artificial membranes and epithelia, e.g. Dainty & House (1966). This therefore leaves little time for any appreciable diffusion into the tissue. This is therefore consistent with agonists acting by setting up a pacemaker in the superficial cells. (It also implies that there is not a layer around the superficial cells which retards the diffusion of these agonists.)

If such a system operated it would explain why the kinetics of antagonists do not appear to be faster when strips are used as against intact pieces of ileum. In addition it might explain the discrepancy observed by Paton & Rang (1965) between the kinetics of onset of atropine’s action and the kinetics of uptake. They found that although at equilibrium atropine appeared to be largely bound to
receptors the rate of occupation of these sites was very much slower than the rate of onset of antagonism. As Thron & Waud (1968) suggested, this would occur if only the most easily accessible cells need be activated by cholinergic agents to produce their effect. The kinetics of atropine's antagonism would therefore reflect only the relatively rapid access of atropine to the most superficial cells, whereas the gradual diffusion through the tissues would be much slower.

However there is another possible implication of a pacemaker system, and this is that in the presence of an antagonist the agonist has to travel further into the tissue before it can set up a pacemaker. Such a pacemaker shift has been demonstrated when carbachol or epinephrine is applied to spontaneously beating guinea-pig atria, West, Falk & Cervoni, (1956). The occurrence of a pacemaker shift might also explain Cuthbert & Dunant's (1970) observation that when an antagonist was present in the bathing solution surrounding an isolated piece of guinea-pig ileum, and its concentration was greater than that of its dissociation constant, the agonist diffusion half times became greater and the transient analysis was therefore no longer valid. As in every preparation the critical concentration at which the transient analysis failed was equal to the antagonist's dissociation constant, and as at these low occupancies the action of the agonist would not be expected to be limited by the rate of dissociation of the antagonist from the receptors, it is possible that above the critical concentration there is a pacemaker shift as a result of which the agonist molecules are no longer able to produce their effect by action on the superficial cells only and so they have to travel deeper into the tissue.

Due to the lack of any precise information concerning either the existence or the behaviour of pacemaker shifts in guinea pig
ileum it is difficult to predict how such shifts might influence the kinetics of drug action. However to account for Paton & Rang’s observations such a shift would have to be small. A shift would however be expected to have little effect on the kinetics of antagonism if the antagonist diffuses relatively quickly through the bulk of the extracellular space, the kinetics of antagonism reflecting principally the rate at which the antagonist concentration in the proximity of the receptors changed to that in the bulk of the extracellular space.

The similarity between the kinetics of antagonists using muscle strips or intact pieces of ileum could therefore be due to agonists acting by a pacemaker mechanism and/or an access-limited model in which the concentration of antagonist rises relatively rapidly throughout the bulk of the extracellular space.
The relationship between the structure and affinity of a drug, its concentration, and the observed rates of antagonism

In the introduction the general relationship between potency and speed was discussed; the tendency for the more potent compounds also to act more slowly. This relationship may however only operate over a threshold value of affinity as it was found that Ph$_2$AOEX$_2$Et appears to act just as fast as penty1 TEA although its affinity is much higher.

In addition other factors must also influence the relative speeds of different antagonists; Paton & Rang (1965) found that although atropine has a lower affinity than methylatropinium, the rate of recovery from atropine was similar to that from methylatropinium. In addition the difference in the rates of onset were not only due to the concentrations of antagonist used as the apparent $k_1$ of atropine, calculated from the rate of onset, was lower than that of methylatropinium. The converse was found by Paton (1961) when investigating the kinetics of alkyl TMA compounds. The variation in the rates of onset appeared to be primarily due to the concentrations used, the apparent $k_1$ values not varying detectably from compound to compound. However the rate of recovery from the alkyl TMA compounds did fall as their affinity increased.

The exact relationship between the structure of a compound and the observed rates of antagonism can not be explained at this stage but they must be linked to various factors involved in the access limitation. It may turn out that the relative rates of antagonists are linked with the properties of the basement membrane and that this layer has an element of selectivity; Brandt (1962) observed that the extraneous coats of the plasma membrane of amoeba could distinguish between various molecular analogues.
In I.1 it was found that when the concentration of BTrMe was increased the observed time constant for recovery became faster. Such an increase would be expected in a limited biophase system as shown by Colquhoun & Ritchie's (1972) Figure 1.

However in I.4 it was found that the rate of recovery from penty1 TEA became slower as the concentration of antagonist was increased and a similar effect was also observed by Paton (1961) following increased concentrations of mepyramine and atropine. This effect could be due to intracellular accumulation, but quaternary compounds such as penty1 TEA are not thought to penetrate cell membranes to an appreciable extent; Del Castillo & Katz (1955) observed that when acetylcholine was applied intracellularly into frog sartorius muscle cells it did not produce a response whereas that applied extracellularly did. This suggests that the intracellularly applied acetylcholine was not able to leave the cells to an appreciable extent. In addition, as discussed by Waddel & Bates (1969), intracellular pH estimations are made using weak acids and bases assuming that the ionised form is not able to cross the cell membrane whereas the unionized is. As such estimations agree with those using intracellular electrodes it seems likely that the ionized form is indeed not able to cross the cell membrane. In addition the rate of recovery from low concentrations of penty1 TEA was not retarded by increasing the time for which the tissue was exposed to the antagonist, which might be expected if the slow recovery was due to intracellular accumulation.

It therefore seems unlikely that the slow rates of recovery following high concentrations of penty1 TEA is due to intracellular accumulation. There does not seem to be any other obvious cause, but perhaps it is linked with the properties of the basement membrane.
CONCLUSION

This study was undertaken to determine whether the kinetics of antagonists on guinea-pig ileum were limited by the rate at which they interact with the receptors. The antagonists BTrMe, lachesine and pentyl TEA were investigated and their kinetic behaviour was not found to be consistent with the predictions of the interaction-limited model based on the receptor model of Stephenson (1956), (see summaries at the ends of Parts I and II).

The discrepancies do not of themselves necessarily indicate that access is rate-limiting as the interaction model might not be appropriate. However it would be difficult to explain the variability of kinetic measurements from one experiment to another if access is not rate limiting. The same applies to the effects of lever, preparation and bathing solution discussed in Part III. In addition the transitional stage observed in I.3 would be difficult to explain. It therefore seems more probable that the discrepancies between the observed kinetics and the prediction of the interaction limited model used in this study are due to access being rate limiting rather than the interaction limited model being inappropriate.

As the rates of onset and offset, when an antagonist is added or removed from the bathing solution, appear to be access limited, values of k₁ and k₂ can not be determined from such kinetic measurements. However the rate of offset of a slow antagonist on superimposition of high concentrations of a fast antagonist may be limited by its rate of dissociation from the receptors, but the possibility can not be ruled out that a different access limitation is then operating.

Two types of access models have been considered in both of which
the rate at which the concentration of a drug changes in the proximity of the receptors is slowed by the binding of the antagonist to the tissue. In the first, the kinetics of antagonism reflect the rate at which the antagonist penetrates into the extracellular space of the tissue. In the second the kinetics reflect the rate at which the antagonist diffuses from the extracellular space to the receptors, perhaps across a barrier such as the basement membrane.

These two models could perhaps be distinguished by the rate at which antagonists diffuse through longitudinal muscle strips; according to the latter model antagonists might be expected to diffuse through muscle strips much faster than the kinetics of antagonism would suggest, whereas according to the former model, penetration would be expected to be initially retarded and more nearly related to the kinetics of antagonism.
APPENDIX
THE USE OF DIFFERENT AGONISTS IN ANTAGONIST AFFINITY CONSTANT ESTIMATIONS

During the course of the study of the kinetics of acetylcholine antagonists on guinea-pig ileum, affinity constant estimations were made using the agonists carbachol and pentyl TMA. TABLE A.1 compares the values of \( \log (K_{aff}) \) obtained using the two agonists in separate independent experiments.

None of the differences between the means is significant according to Student's t test for independent samples. Nevertheless, in each case pentyl TMA gave a higher apparent affinity and so there could be a real small difference between the means which would not be statistically significant due to the variance of the observations.

Affinity constant estimations were therefore made using pentyl TMA and carbachol alternately in the same experiment as shown in DIAGRAM A.2. TABLE A.3 compares the values of \( \log (K_{aff}) \) obtained using the two agonists alternately in the same experiment. In every case the value of affinity from the pentyl TMA responses was larger than that obtained from the carbachol responses.

The significance of the difference between the means can be exemplified by the sign test: if the difference between two readings of a pair is equally likely to be positive or negative, the probability of 22 positive results or 22 negative results would be \( \frac{2}{2^{22}} \approx 0.00004 \), i.e. is very unlikely to occur.

In order to investigate the possibility that the differences in apparent affinity were due to differences in stimulation of the ganglia by the agonists, the effect of hexamethonium was investigated.

As \( 2.76 \times 10^{-4} \) M hexamethonium bromide had been present in all experiments, affinity constant estimations were made of this concentration
TABLE A.1: Mean values of $\log K_{\text{aff}}$ S.E.M. using antagonist concentrations in the ranges indicated, and using carbachol and pentyl TMA in separate experiments.

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>Concentration Range M</th>
<th>Mean log $K_{\text{aff}}$ S.E.M.</th>
<th>Carbachol</th>
<th>Pentyl TMA</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>BTlMe</td>
<td>$6 - 200 \times 10^{-10}$</td>
<td>$10.129 \pm 0.044$ (27)</td>
<td>TABLE I.15</td>
<td>$10.206 \pm 0.068$ (9)</td>
<td>$&gt;0.2$</td>
</tr>
<tr>
<td></td>
<td>$10 - 39 \times 10^{-10}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lachesine</td>
<td>$0.5 - 2 \times 10^{-8}$</td>
<td>$8.817 \pm 0.027$ (26)</td>
<td>TABLE II.2</td>
<td>$8.963 \pm 0.011$ (5)</td>
<td>$&gt;0.2$</td>
</tr>
<tr>
<td>Pentyl TMA</td>
<td>$0.3 - 8 \times 10^{-4}$</td>
<td>$4.558 \pm 0.016$ (24)</td>
<td>TABLE I.19</td>
<td>$4.593 \pm 0.029$ (5)</td>
<td>$&gt;0.2$</td>
</tr>
</tbody>
</table>

Details
Preparation - intact pieces of ileum
Bathing solution - Tyrode's
Lever - isotonic
(n) - number of estimations, each using ileum from a different guinea-pig
P - the probability of observing the difference in apparent affinity, determined by Student's t test for independent samples

![Diagram A.2](image)

DIAGRAM A.2: The method used to estimate the affinity constant of an antagonist using carbachol and pentyl TMA alternately in the same experiment.

Details
Experiment 73
Preparation - intact pieces of ileum
Bathing solution - Tyrode's
Lever - isotonic
Antagonist - $50 \times 10^{-7}$ M Ph$_2$ACMe$_2$Et
Agonists - carbachol, $1 \times 10^{-7}$ M and $2 \times 10^{-7}$ M (in the absence of antagonist) $200 \times 10^{-7}$ M and $400 \times 10^{-7}$ M (in the presence of the antagonist)
- pentyl TMA, $1.5 \times 10^{-6}$ M and $3 \times 10^{-6}$ M (in the absence of antagonist) $300 \times 10^{-6}$ M and $600 \times 10^{-6}$ M (in the presence of the antagonist)
C - responses produced by carbachol
The change in slope is discussed on P.152.
<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Antagonist Concentration M</th>
<th>Log $K_{a}$ff</th>
<th>d</th>
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<tr>
<td></td>
<td></td>
<td>Carbachol</td>
<td>Penty TMA</td>
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<tr>
<td>41</td>
<td>$10 \times 10^{-10}$</td>
<td>10.000</td>
<td>10.079</td>
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<td>42</td>
<td>9.778</td>
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<td>43</td>
<td>10.146</td>
<td>10.230</td>
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<td>Pentyl TMA</td>
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<td>43</td>
<td>$2.5 \times 10^{-4}$</td>
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<td>203 I</td>
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<td>Ph 2AGMe Et</td>
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<td>54</td>
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<td>$26 \times 10^{-7}$</td>
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<td>55</td>
<td>7.691</td>
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<td>7.668</td>
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<td>57</td>
<td>7.534</td>
<td>7.835</td>
<td></td>
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</tbody>
</table>

$d = \log K_{a}$ff (from the penty TMA responses) - $\log K_{a}$ff (from the carbachol responses)

Details
Preparation - intact pieces of ileum
Bathing Solution - Tyrode's
Lever - isotonic
Antagonists- as indicated
Agonists - carbachol and penty TMA
of hexamethonium using pentyl TMA and carbachol alternately in the same experiment. These results, shown in TABLE A.4, do not indicate that hexamethonium can distinguish between carbachol and pentyl TMA.

In addition the dose ratio produced by $50 \times 10^{-7} \text{M} \text{Ph}_2\text{AOEt} \text{Me}_2 \text{Et}$ was determined in the absence of hexamethonium and when hexamethonium was present throughout. The results of these experiments are shown in TABLE A.5. In every case the estimate of affinity from the pentyl TMA responses was larger than that using the carbachol responses. As the mean difference in the presence of hexamethonium was 0.264 while that in its absence was 0.207 there is no reason to believe that the difference between carbachol and pentyl TMA is due to the extent to which they stimulate the nicotinic receptors of the ganglia.

Affinity constant estimations were also made using partially and totally denervated muscle strips. The extent of the denervation of the strips was estimated after staining with methylene blue and by whether a response was produced by the specific ganglia stimulant, $\text{NH}_2\text{C}_6\text{H}_4\text{CH}_2\text{CH}_2\text{N}^+\text{Me}_3 \text{I}^-$. All the preparations used in TABLE A.6 had less than half of their surface covered by the nerve network and were obtained using animals of the normal weight range, i.e. 150-400g. The animals used in TABLE A.6 were above 500g because of the difficulty in obtaining totally denervated preparations from smaller animals.

When partially denervated longitudinal muscle strips were used instead of intact pieces of ileum, the mean difference between carbachol and pentyl TMA appeared to be smaller than when intact pieces of ileum were used, as shown in TABLE A.6 and also DIAGRAM A.7.

When totally denervated strips were used, of four estimations made, in one case using carbachol gave a higher apparent affinity than pentyl TMA, and in one case no difference was observed. The probability
TABLE A.4: The dose ratio produced by $2.76 \times 10^{-4}$M hexamethonium bromide, using carbachol and pentyl TMA alternately in the same experiment

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>DR</th>
<th>Carbachol</th>
<th>Pentyl TMA</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td>69</td>
<td></td>
<td>1.20</td>
<td>1.30</td>
<td>+0.1</td>
</tr>
<tr>
<td>70</td>
<td></td>
<td>1.43</td>
<td>1.02</td>
<td>-0.41</td>
</tr>
<tr>
<td>72</td>
<td></td>
<td>1.22</td>
<td>1.07</td>
<td>-0.15</td>
</tr>
<tr>
<td>73</td>
<td></td>
<td>1.02</td>
<td>1.09</td>
<td>+0.07</td>
</tr>
</tbody>
</table>

$d = DR (\text{from the pentyl TMA responses}) - DR (\text{from the carbachol responses})$

TABLE A.5: Values of $\log K_{\text{aff}}$ of $\text{Ph}_2\text{AgMe}_2\text{Et}$ ($50 \times 10^{-7}$) using carbachol and pentyl TMA alternately in the same experiment either in the absence of hexamethonium or with $2.76 \times 10^{-4}$M hexamethonium present throughout

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Log $K_{\text{aff}}$</th>
<th>In the absence of Hexamethonium</th>
<th>In the presence of Hexamethonium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Carbachol</td>
<td>Pentyl TMA</td>
</tr>
<tr>
<td>69</td>
<td></td>
<td>7.292</td>
<td>7.881</td>
</tr>
<tr>
<td>70</td>
<td></td>
<td>7.589</td>
<td>7.719</td>
</tr>
<tr>
<td>72</td>
<td></td>
<td>7.584</td>
<td>7.619</td>
</tr>
<tr>
<td>73</td>
<td></td>
<td>7.668</td>
<td>7.740</td>
</tr>
</tbody>
</table>

$d = \log K_{\text{aff}} (\text{from the pentyl TMA responses}) - \log K_{\text{aff}} (\text{from the carbachol responses})$

Details of TABLES A.4 and 5
Preparation - intact pieces of ileum
Bathing solution - Tyrode's
Lever - isotonic

TABLE A.6: Values of $\log K_{\text{aff}}$ of $\text{Ph}_2\text{AgMe}_2\text{Et}$ ($50 \times 10^{-7}$) using carbachol and pentyl TMA alternately in the same experiment. Partially denervated muscle strips were used in the presence of hexamethonium

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Log $K_{\text{aff}}$</th>
<th>Carbachol</th>
<th>Pentyl TMA</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td>65</td>
<td></td>
<td>7.563</td>
<td>7.631</td>
<td>+0.068</td>
</tr>
<tr>
<td>67</td>
<td></td>
<td>7.428</td>
<td>7.486</td>
<td>+0.058</td>
</tr>
<tr>
<td>74</td>
<td></td>
<td>7.567</td>
<td>7.609</td>
<td>+0.042</td>
</tr>
<tr>
<td>75</td>
<td></td>
<td>7.458</td>
<td>7.560</td>
<td>+0.102</td>
</tr>
</tbody>
</table>

Details
Preparation - partially denervated muscle strips
Bathing solution - Tyrode's
Lever - isotonic
Diagram A.7: Estimate of the affinity of Ph$_2$AOEMe$_2$Et using carbachol and pentyl TMA alternately in the same experiment, and also using partially denervated longitudinal muscle strips.

Details
Experiment 67
Preparation - partially denervated muscle strips
Bathing solution - Tyrode's
Lever - isotonic
Agonist - 40 x 10^{-7} M Ph$_2$AOEMe$_2$Et
Antagonist - 40 x 10^{-7} M Ph$_2$AOEMe$_2$Et
Carbachol, 2 x 10^{-7} and 4 x 10^{-7} M (in the absence of antagonist)
300 x 10^{-7} and 600 x 10^{-7} M (in the presence of the antagonist)
Pentyl TMA, 3 x 10^{-6} and 6 x 10^{-6} M (in the absence of antagonist)
900 x 10^{-6} and 450 x 10^{-6} M (in the presence of the antagonist)
C - responses produced by carbachol
The change in slope is discussed on p. 152

Table A.8: Values of log $K_{aff}$ of pentyl TEA using carbachol and pentyl TMA alternately in the same experiment. Totally denervated muscle strips were used and no hexamethonium was present.

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Antagonist Concentration M</th>
<th>Log $K_{aff}$</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carbachol</td>
<td>Pentyl TMA</td>
<td>d</td>
<td></td>
</tr>
<tr>
<td>202 XX</td>
<td>2.5 x 10^{-4}</td>
<td>4.526</td>
<td>4.530</td>
<td>+.004</td>
</tr>
<tr>
<td>203 II</td>
<td>4.651</td>
<td>4.653</td>
<td>+.002</td>
<td></td>
</tr>
<tr>
<td>202 I</td>
<td>15 x 10^{-4}</td>
<td>4.456</td>
<td>4.473</td>
<td>+.017</td>
</tr>
<tr>
<td>202 II</td>
<td>4.621</td>
<td>4.596</td>
<td>-.025</td>
<td></td>
</tr>
<tr>
<td>203 II</td>
<td>4.444</td>
<td>4.444</td>
<td>+.000</td>
<td></td>
</tr>
</tbody>
</table>

Details
Preparation - totally denervated muscle strips
Bathing solution - Tyrode's (no hexamethonium present)
Lever - isotonic
of these differences occurring by chance, according to the sign test is between .625 and .156, according to whether 'no' difference is counted as a positive or a negative.

Discussion

Abramson, Barlow, Mustafa & Stephenson (1969) compared the affinity constants of several antagonists using the agonists carbachol, acetylcholine, pentyl TMA and ethoxethylltrimethylammonium, in separate experiments. They concluded, using Student's t test for independent samples, that there was no significant difference. However using independent samples differences in apparent affinity as large as 0.2 log units might not be 'significant' due to the variance of the observations. The largest difference in mean log affinity that they observed was in the one comparison they made between carbachol and pentyl TMA for the antagonist phenylpentylethylpyrrolidinium. The mean log affinity using carbachol was 5.650 ± 0.036 (6), and using pentyl TMA 5.720 ± 0.020 (6). This is consistent with the results found here.

The difference in apparent affinity between using pentyl TMA and carbachol is unlikely to be due to the agonists altering the antagonist affinity by interacting with the receptors because the difference was observed using the two agonists alternately in the same experiment.

The difference in apparent affinity is also unlikely to be due to differences in stimulation of the nicotinic receptors in the ganglia, because the difference was observed when hexamethonium was present throughout and did not appear to increase in the absence of hexamethonium. In addition the dose ratio produced by hexamethonium
did not appear to be affected by whether carbachol or pentyl TMA was used.

The decrease in, or lack of, difference observed when totally denervated strips were used, is unlikely to be due to using longitudinal muscle strips rather than intact pieces of ileum because a consistent, though small, difference was observed when partially innervated muscle strips were used.

It therefore seems likely that the difference in apparent affinity is due to differences in the extent to which carbachol and pentyl TMA stimulate receptors in the ganglionic layer before the antagonist is added. These receptors can not be of the traditional nicotonic variety because they do not appear to be blocked by hexamethonium and they can not be of the traditional muscarinic variety because of the difference in apparent antagonist affinity observed.

It is not clear whether the muscarinic antagonists block these anomalous receptors directly or indirectly via the postganglionic receptor.

It is also not clear whether part of the action of carbachol, in the absence of the antagonists, is due to stimulation of ganglionic receptors. A very large number of affinity constant estimations would have to be made using denervated muscle strips and intact pieces of ileum before any genuine difference would be expected to be 'detected'. This is because of the very small size of the possible difference compared with the variability of the observations.

Alternatively, ganglionic stimulation of this sort could be demonstrated if an agonist was found which gave values of apparent affinity lower than those obtained using carbachol.
However part of the action of pentyl TMA, in the absence of antagonist, appears to be due to stimulation of ganglionic receptors. Therefore any affinity measurements made using this agonist and intact pieces of ileum or longitudinal muscle strips in which the ganglionic layer has not been removed, are likely to overestimate the true value of log $K_{eff}$ by up to about 0.2 log units.

Ganglionic stimulation might account for differences observed by Furchgott & Burzstyn (1967), and Waud (1969) in affinity estimates of partial agonists using different methods. If the partial agonist stimulated ganglionic receptors, comparisons of dose-response curves before and after treatment with an irreversible antagonist would be expected to yield, as was found, higher values of apparent affinity than if the partial agonist is used as a competitive antagonist after treatment with an irreversible antagonist, or comparison of its dose response curve with that of carbachol. Similarly the apparent affinity of the partial agonist in the last method would be expected to vary with the relative potencies of the partial and full agonist at these ganglionic receptors. The sizes of the differences expected would be too small to be 'statistically' significant. Nevertheless it would be interesting to compare the various methods using totally denervated muscle strips.

'Muscarinic' ganglionic receptors could also account for Burgen & Hiley's (1974) finding of two populations of acetylcholine receptors in guinea-pig ileum, one with an affinity for acetylcholine of $1.6 \times 10^{-8} M$ and the other of $1.6 \times 10^{-6} M$, both being present in roughly equal amounts. This observation could be due to their using homogenates of longitudinal muscle without considering whether the ganglion layer had been removed or not.
THE INCREASE IN THE SLOPE OF THE LOG DOSE-RESPONSE CURVE IN THE
PRESENCE OF \( \text{Ph}_2\text{ACOEMA}_2\text{Et} \)

In every experiment in which the affinity of this antagonist
was estimated using the alternating technique, (see DIAGRAMS A.2 and 7),
a n increase in slope of the log dose-response curve was observed, even
when totally denervated preparations were used. The change appeared
to increase as the concentration of antagonist increased, (a concentration
range between \(13 \times 10^{-7}\) and \(50 \times 10^{-7}\) M was investigated), and the
change in slope did not appear to be effected if pentyl TMA or hexyl TMA
was used instead of carbachol.

A similar effect was noted by Guarino & Bovat (1949). They
observed that the synthetic curare derivative 2559 F (tri-iodoethy late of
tri(-β-diethylaminoethoxy)-1,2,3-bensene) caused a steepening of the
acetylcholine log dose-response curve using the frog rectus abdominis
preparation.

There seems to be no obvious explanation within the framework
of the classical theory of competitive drug antagonism.

(As the change in slope is small, the dose ratios produced by
various concentrations of this antagonist, e.g. TABLE A.3, were
determined assuming a parallel shift in the log dose-response curve.)
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