The Pharmacology of Homologous Series

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1. Introduction

It is the common practice of organic chemists who are concerned with the discovery of new drugs to take some compound which is known, or has been unexpectantly found, to possess some interesting or clinically desirable pharmacological properties, as a model, and to study the effect of making relatively small changes in its structure. The simplest chemical change that can be made is to alter the size of an alkyl group or of a polymethylene chain; in other words to investigate the pharmacology of a homologous series. An investigation of this type not only has practical advantages, such as ease of preparation of homologues, the small changes in physical and chemical properties involved, etc. but also it has frequently led to the discovery of highly active drugs. Moreover there is the theoretical likelihood that a detailed study of homologous compounds may reveal mechanisms of drug action which could not be revealed by a study of drugs differing more fundamentally in structure.

The difficulty of reviewing the pharmacology of homologous series resides in the fragmentary information in the literature, simply because, once a highly active member of a homologous series has been discovered, most research workers lose all interest in the rest of the series. Practical considerations override theoretical considerations; this is natural, since the chemist is primarily concerned with the discovery of new drugs. At the same time there are a few workers who are more interested in the mechanisms of drug actions than in the discovery of new therapeutic agents.

Pharmacology began as an attempt to put therapeutics on a scientific basis, but it was probably A. J. CLARK [1][1]) who first claimed that it was an independent scientific subject, concerned with the actions of chemical substances on living cells. This article will be written from the Clark point of view.

The general plan of the article will be, firstly, to define the meaning of the term 'homologous series' and to describe briefly how physicochemical properties change as series are ascended; secondly, to classify, so far as that is possible, the several ways in which pharmacological properties change, qualitatively or quantitatively, as homologous series are ascended; thirdly, to consider, in the light of this classification, particular groups of drugs for which adequate data on homologous compounds are available. Finally some comments will be made on the pharmacology of 'hybrid homologous series' (see p. 308 for definition) since such series, although not truly homologous, are of considerable importance in chemical pharmacology.

Any review of this type is bound to be selective, and limited to some extent by the particular interests of the author. No chemist interested in synthetic drugs, and no pharmacologist, can be expected to be familiar with the whole of the vast literature of pharmaceutical chemistry and pharmacology; moreover the particular aspect with which the author is concerned is not indexed as such in abstract journals. Consequently the author may well have overlooked im-

[1]) The numbers in brackets refer to References, page 338.

portant contributions in fields of knowledge unfamiliar to him; at the same time, he does not think that the multiplication of examples necessarily increases understanding. On the contrary, he thinks that a real understanding of a few examples of how pharmacological activity varies within homologous series may illuminate the general problem, and give workers in highly specialized fields some guidance, and even some hints, about the most profitable compounds to prepare and investigate.

1.1 *Definition of Homologous Series*

Homologous series of organic compounds are usually defined as derivatives of paraffin hydrocarbons, C_nH_{2n+2}, such that each member of the series differs from the next higher member by containing one methylene group less, and the series can, at least in theory, be extended indefinitely.

On the basis of this definition, two types of homologous series can be distinguished: (i) *Alkane series* of the general formula, $C_nH_{2n+1}X$, where X is the radical or complex structure which defines the series; and (ii) *polymethylene series* of general formula, $X(CH_2)_nY$, where X and Y are the same or different radicals.

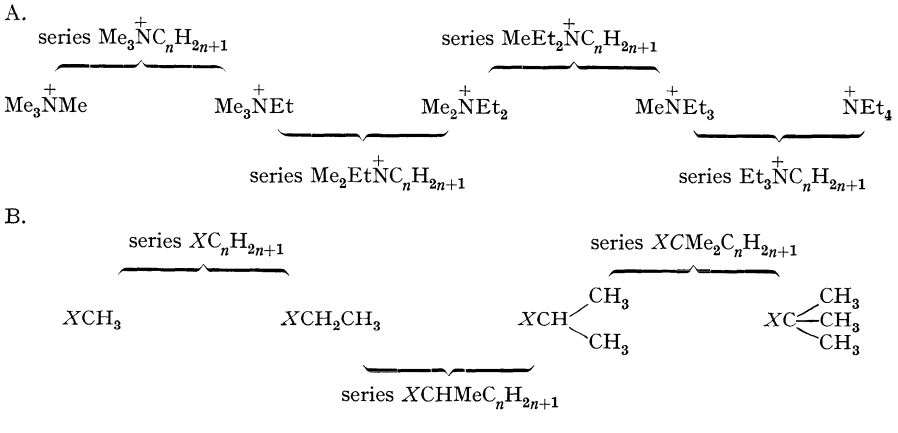


Figure 1
Hybrid Series.

A third type of homologous series, of less interest to pharmacologists, is the *cyclo*polymethylene series, i.e. compounds of the general formulae, $(CH_2)_n{>}X$ or $(CH_2)_n{>}CHX$, in which the radical X may either form part of the ring or may be attached to one of the C-atoms of the ring.

It is important to notice that a series of organic compounds, each member of which differs from the next higher member by one methylene group, is not necessarily a homologous series; for example, series A and B (Figure 1) are not true homologous series, although each successive member, except the first, belongs to two true homologous series.

Series of types A and B are of great importance in chemical pharmacology and will be referred to as *hybrid series*. For example, it frequently happens that an *iso*propyl group confers higher activity in some group of compounds than an *n*-propyl group, so that the chemist is apt to regard the *iso*propyl group as homologous with the methyl and ethyl groups, whereas it is only homologous with the latter, which is the first member of a branched chain series $X\text{CHMeC}_n\text{H}_{2n+1}$, that has different spatial characteristics from the straight chain series. In this article a clear distinction will be made between true and 'hybrid' homologues.

1.2 *Physicochemical Properties*

In the alkane series, $\text{C}_n\text{H}_{2n+1}X$, apart from the first one or two members ($n=0$ or 1) which may display exceptional properties, the physicochemical properties of the group X remain more or less constant throughout the series. For example, in the series $\text{C}_n\text{H}_{2n+1}\text{CO}_2\text{H}$ the acidic dissociation constants ($ka^{25°}$) are of the same order ($1{\cdot}0$–$2{\cdot}0\times 10^{-5}$) for $n=1$ to 8, but formic acid ($n=0$) has a dissociation constant about ten times greater ($2{\cdot}1\times 10^{-4}$). A more pharmacologically interesting series of the same type would be the series $C_n\text{H}_{2n+1}\text{N}R_2$ (where $R=\text{H}$ or a small alkyl group), but data on dissociation constants for a reasonably long series do not appear to be available.

The more general physicochemical properties of the alkane series increase or decrease in a regular manner as the series is ascended; in particular, all those properties which depend upon an equilibrium between two phases and are measured when equilibrium is established, increase or decrease geometrically as the number of C-atoms increases arithmetically, so that the logarithms of the numerical values of such properties are directly proportional to the number of C-atoms. Physicochemical properties which obey this rule include solubility, vapour pressure, capillary action, partition coefficients, etc. The rule is a direct consequence of the constant increment in free energy for the addition of each methylene group. At the same time new properties may appear as an alkane series is ascended. One of the most important of these new properties, from a pharmacological point of view, is micelle formation, particularly in aqueous solution. Owing to the polar nature of the water molecule, alkane derivatives containing a hydrophilic group X tend to form bundles of molecules in which the hydrophobic paraffin chains, attracted to each other by van der Waals forces, form an inner core surrounded by the hydrophilic groups in intimate association with water molecules. This micelle formation, which only becomes important in compounds containing hydrocarbon chains of twelve or more carbon atoms, confers detergent-like properties upon solutions of alkane derivatives, since the hydrocarbon cores of the micelles can take lipophilic substances into solution; at the same time it reduces the effective concentration of single molecules of the solute, which exist in equilibrium with the micelles.

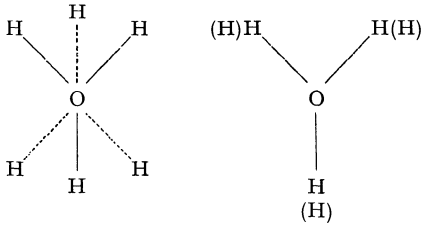
The physicochemical properties of polymethylene series, $X(\text{CH}_2)_nY$, do not change in so simple and predictable a manner as those of the alkane series. In

particular, the properties of the terminal groups X and Y may be influenced by their greater or less proximity to each other, especially if they have strong polar character. For example, the first dissociation constants (ka_1) of the aliphatic dibasic acids, $CO_2H(CH_2)_nCO_2H$, decrease a thousandfold from oxalic acid ($n=0$; $ka_1=3{\cdot}5\times10^{-2}$) to adipic acid ($n=4$; $ka_1=3{\cdot}7\times10^{-5}$) and then remain approximately constant at about 3×10^{-5} for higher members, at least up to sebacic acid ($n=8$). The second dissociation constants (ka_2) show a less striking variation, but the ratio ka_1/ka_2 decreases from about 900 for oxalic acid to about 10 for adipic and higher acids of the series.

Some polymethylene series, particularly those in which the terminal groups are both acidic or both basic, show a remarkable alternation of some physical properties as the series are ascended, but this alternation appears to be confined to properties, like melting-points and solubilties, which depend upon an equilibrium between the solid substance and a liquid phase. Thus in the dibasic acid series, $CO_2H(CH_2)_nCO_2H$, the melting points of the acids containing an odd number of carbon atoms are consistently lower than those of neighbouring acids with an even number of carbon atoms. Similarly acids with an odd number of methylene groups are much more soluble in water than acids with an even number of methylene groups.

1.3 *Conformation of Hydrocarbon Chains*

The conformation of an alkyl group or of a polymethylene chain may be defined as any one of a theoretically infinite number of arrangements of its atoms in space, the multiplicity of possible arrangements arising from the assumed possibility of free rotation about the axis of single C–C bonds. However, the number of probable conformations is severely restricted by repulsive forces



(A) *trans* or staggered (B) *cis* or eclipsed

Figure 2

Views down the C–C bond of ethane.

between non-bonded atoms, these repulsive forces being a high exponential function of any interatomic distance less than the sum of the van der Waals radii of the atoms concerned. In ethane, for example, the most probable conformation is the *trans* or staggered form A (Figure 2) and the least probable the *cis* or eclipsed form B. In a paraffin chain the maximum distance between two

carbon atoms in the 1:4 positions relative to each other is 4·06 Å in the *trans* arrangement; this distance is twice the sum of the van der Waals radius of a methyl group (2 Å) so that any conformation of the hydrocarbon chain other than the fully *trans* or staggered form will be hindered by repulsive forces between non-bonded atoms. A very large number of other conformations may occur momentarily, but there will be a probability distribution of conformations in which relatively few conformations will predominate. Thus in *n*-butane (Figure 3) the conformation C (*trans* or staggered) will predominate, conforma-

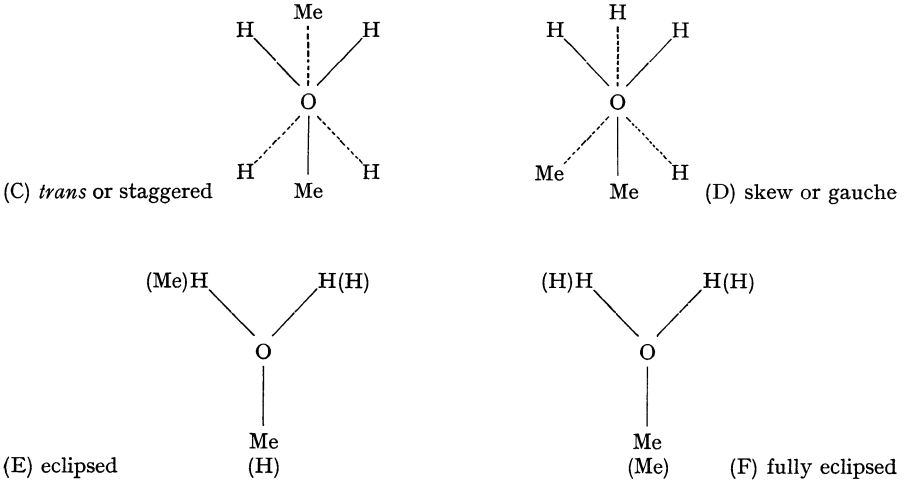


Figure 3
Views down the central C^2–C^3 bond of n-butane.

tions D (two enantiomorphous skew or gauche forms), E (eclipsed) and F (fully eclipsed) decreasing in probability of occurrence in that order. It has been calculated that the energies of the fully eclipsed (F), the eclipsed (E) and the skew (D) conformations of *n*-butane are about 3·6, 2·9, and 0·8 kcal per mole respectively greater than that of the staggered form (C). The number of energetically preferred conformations naturally increases rapidly with the number of carbon atoms in the hydrocarbon chain, and the probability distribution of conformations for chains of more than four carbon atoms can only be derived by somewhat tedious mathematical calculation.

Two out of many other factors influencing the probability of particular conformations may be mentioned: (i) crowding of alkyl groups attached to a single C- or N-atom may lead to bond-angle deformation—e.g. in tetraethylammonium crystallographic data indicate that the N–C–C-angle is nearer to 120° than to the normal angle, 109° 28′; and (ii) adsorption of a molecule at an interface,

e.g. at a cell membrane, may facilitate conformations unlikely to occur to an appreciable extent in simple solution.

The term 'conformation', originally referring to an infinite number of arrangements, has come to refer mainly to the most probable or energetically preferred conformations. The term 'rotational isomers', commonly used in the literature of chemical physics, is in some ways a more illuminating term than 'conformation', since it implies a limited number of arrangements of the atoms of a molecule in space arising from the rotation of single C–C bonds; at the same time it has the disadvantage that the word 'isomer' is associated with a more or less stable substance, whereas the energy barriers between rotational isomers (conformations) are usually too low to permit the isolation of any one of them in a pure state. In the German language the word 'Konstellation' is used in the same sense as the English term 'conformation'.

The importance of conformational factors in chemical pharmacology has only been realized in recent years, although for a long time it has been well-known that the addition of one methylene group to either an alkyl group or a polymethylene chain of a drug molecule may lead to dramatic changes in potency, or even in the type of pharmacological response elicited by the modified drug. Since the general viewpoint of pharmacologists on drug action is that the majority of drugs form reversible complexes with receptors (probably macromolecular constituents of living tissues), the vague idea of fit between the drug and a hypothetical receptor molecule has needed the concept of conformation of drug molecules in order to give precision to the general viewpoint.

Conformational analysis in organic chemistry has been extremely profitable in saturated polycyclic systems, but little accurate information is available about polymethylene chains or about alkyl groups attached to O-or N-atoms. The latter groups of compounds are of particular importance to the chemical pharmacologist. It is sometimes possible to foresee what the preferred conformation will be; e.g. that of an N-*iso*propyl group will be one in which the two methyl groups and the H-atom of the *iso*propyl group are staggered with respect to the two other atoms attached to the N-atom, but this conclusion is really guess-work and urgently needs confirmation by physical methods. Similarly the conformation of polymethylene drugs of the general type $X(CH_2)_nY$ needs detailed analysis. Reliance upon conventional structural formulae or upon atomic models is inadequate; it is recognized that conventional pictures of organic chemical molecules are merely a kind of shorthand, useful to the organic chemist, but it is not so commonly understood that atomic models can only provide information within the framework of the data upon which the models were constructed; whenever the model fails to incorporate important physical factors, it renders conclusions drawn from it unreliable. This is particularly true of relatively small molecules, which include most important drugs; the real value of atomic models resides in enabling the chemist to think about macromolecules, like polypeptides, too complex for him to visualize. In the author's opinion the value of atomic models of small drug molecules has been over-rated.

1.4 *Pharmacological Properties*

The pharmacological properties of homologous compounds change in many and, frequently, unexpected ways as series are ascended, but the following general types of change are commonly encountered:

(1) Activity may increase regularly (e.g. geometrically) as a series is ascended until a maximum is reached for one member, higher members being almost or entirely inactive.

(2) Activity may increase irregularly (i.e. according to no simple mathematical formula) as a series is ascended, reach a maximum value and then decrease, again irregularly. In such series, there may be a range of members with moderate to high activities, or high activity may be confined to one or two members only, higher and lower homologues being only weakly active.

(3) Activity may increase (or decrease) as a series is ascended, reach a relatively high (or low) value and then remain more or less constant for a few or many higher members.

(4) Activity may alternate as a series is ascended, members with an odd number of carbon atoms in an alkyl or polymethylene chain being consistently more active than neighbouring members with an even number of carbon atoms in the chain, or *vice versa*.

(5) The kind of pharmacological property may change as a series is ascended, lower members having one type, and higher members a different type, of predominant action. A common example of this qualitative change within a series is that higher members may antagonize the pharmacological effect of lower members, or *vice versa*.

Since few drugs have only one kind of pharmacological effect, it is not surprising that homologous series may display different types of variation for each of their several kinds of activity. Consequently, it will be convenient to consider homologous drugs in terms of the five types of change listed above, instead of discussing them in terms of the predominant pharmacological class to which they belong. At the same time, within each general type (1)–(5) it may be useful to consider alkane and polymethylene series separately, since the latter series present a different, and somewhat more difficult, problem, even in the prediction and interpretation of their physicochemical properties.

No attempt will be made to discuss all classes of homologous drugs. The author's aim is not to produce a catalogue for reference, but to attempt, by selecting groups of drugs of which he has some knowledge, to interpret the ways in which pharmacological activities change within homologous series; even this aim is probably too ambitious in the present state of knowledge.

Some attention will also be given to the specificities of homologous compounds, either as substrates or inhibitors of particular enzymes, although the author cannot claim to be an authority in this field. The biochemist, particularly when he is using enzyme preparations *in vitro*, is dealing with systems simpler than most of those which the pharmacologist encounters; but not only do some drugs owe

their main pharmacological activity to the inhibition of enzymic reactions but also enzymes provide the pharmacologist with the nearest analogy to his hypothetical 'drug receptors', which he thinks of as macromolecular structures, mainly protein in character, capable of initiating a succession of biochemical events once they have formed some chemical combination, however loose and transient, with relatively small and foreign molecules—in fact, with drug molecules.

The receptor theory of drug action was originally suggested by LANGLEY, but is usually associated with PAUL EHRLICH, who thought in more chemical terms, and however naive his ideas may seem to us now, they have dominated pharmacology and chemotherapy ever since. The discovery of hormones, and particularly of the chemical transmission of the effects of nerve impulses to muscles, ganglia and glands, made it inevitable that pharmacologists should think in terms of the impact of relatively small molecules upon the complex macromolecular structures in living tissues. This impact may involve several types, or combination of types, of chemical interaction; for example it may involve true covalent linkages, ionic or polar forces, or van der Waals (or London) forces. The nature of the hypothetical receptors, except when they happen to be identifiable enzymes, is unknown, but the assumption that receptors exist, that there are specialized areas or, as A. J. CLARK called them, active patches, particularly on cell membranes, is an inescapable conclusion from the immense body of knowledge about the structural and stereochemical specificity of drugs.

At the same time, pharmacological measurements of drug activities cannot be accepted as uncomplicated estimates of drug-receptor interactions. In estimating the relative activities of members of homologous series, the pharmacologist must always bear in mind several complicating factors: such as the rate of access of the drug to, and the rate of escape of the drug from, its ultimate site of action (these rates are unlikely to be equal and cannot usually be simply related to such properties as lipoid solubility, etc.); also the rates of metabolism of members of homologous series may bear no simple relation to their intrinsic activities; this is particularly important in measurements of pharmacological activities in intact animals; for example, in measurements of toxicities. Moreover the administered drug may not be the pharmacologically active molecular species; the latter may sometimes be a metabolite of the original drug, so that, even within homologous series, the measured activity may be a complex function of the rate of conversion of the administered drug into its pharmacologically active metabolite, the rate of penetration of the latter to its site of action and its intrinsic activity. Consequently the crude estimates of the activities of members of homologous series, however helpful they may be in the discovery of clinically useful drugs, rarely provide uncomplicated information about the way in which intrinsic activities change from one member of a homologous series to another. At the same time these estimates present a challenge, particularly to the chemist who cannot believe that drug action will ever be explained in other than chemical terms.

2. Particular Examples of Homologous Series

As already explained, I shall make no attempt to deal in detail with the vast number of homologous series which have been more or less carefully investigated. Series will be chosen as representative of the several ways (listed in Section 1.4) in which activities vary within them. This choice is designed to concentrate attention upon the general problem of the pharmacology of homologous series, and is bound to reflect the personal interests of the author.

The first group of drugs to be considered is that for which a geometrical increase in activity occurs as a series is ascended. Drugs of this type are frequently referred to as 'structurally non-specific', but this is an unfortunate term because it is only certain kinds of chemical compounds which display this regularity; all that the term means is that pharmacological activities vary within the series in the same way as some physicochemical properties, the term structural specificity being usually reserved for series in which one or two members show exceptional activity.

The second group of homologous drugs to be considered includes those series in which one or two members display exceptional activity, such that no simple physicochemical explanation can be given. This group, which includes the majority of homologous drugs, presents the most difficult and challenging problem in chemical pharmacology, and has compelled us to think in terms of a unique fit between the drug and the hypothetical receptor upon which it is supposed to have its effect. Among this group we must also consider series in which only one member has activity.

The third group of homologous series deals with drugs which display alternating activities.

The fourth group of homologous series deals with series in which the length of an alkyl or polymethylene chain determines the *kind* of pharmacological response. Common examples are provided by series in which higher members antagonize the pharmacological effects of lower members or *vice versa*. This reversal effect is of great interest in theories of drug action, particularly because it frequently happens that certain intermediate members, called 'partial agonists', produce either positive or antagonistic effects according to dosage, so that some authors have distinguished between the affinity of a drug for its receptor and the 'intrinsic activity' or 'efficacy' of the drug-receptor combination. PATON [2] has suggested a new approach to this problem; his idea is that pharmacological activity is determined by the *rate* of combination of drugs with receptors, whereas occupation of receptors necessarily involves their inactivation and consequently blockage of the pharmacological effect. It seems to the author that PATON's theory can only mean that an 'activated drug-receptor' unit is the origin of a pharmacological effect, whereas a more stable drug-receptor combination of lower energy puts the receptor out of action; consequently the rate of dissociation of the drug-receptor complex determines the liberation of receptors free to respond with fresh drug molecules.

Lastly, a few examples of *cyclo*polymethylene drugs will be considered.

2.1 *Geometrical Increase in Activity Within Series*

The simplest way in which biological activities change within homologous series is that molar activity increases geometrically as the number of C-atoms in an alkyl group increases arithmetically. In other words, activity increases as $1:n:n^2:n^3\ldots$, or equiactive concentrations decrease as $1:1/n:1/n^2:1/n^3\ldots$, as a series is ascended, so that there is a linear relation between the logarithm of activity (best measured as equiactive molar concentrations) and the number of methylene groups in an alkane series. Moreover the value of n varies for different homologous series over the remarkably narrow range 2·5–3·3, so that the rth member of a series which obeys this rule will produce some standard biological effect at a molar concentration of about $1/3^{r-1}$ of that of the first member; for example, CLARK [3] found that the concentrations of methanol and n-dodecanol producing equal depression of the isolated frog heart were in the ratio 1:1/266000, i.e. approximately $1:1/3^{12-1}$.

This remarkable regularity is confined to alkane series of drugs which can be roughly classified as 'biological depressants'; this group includes some hypnotics, general anaesthetics, some disinfectants and some volatile insecticides, and is frequently referred to as being structurally non-specific. Actually the group X, which defines the series $C_nH_{2n+1}X$, is not irrelevant, since it is only certain types of organic compounds which display, as biological depressants, the regularity that we are discussing; these series include hydrocarbons, halogenated hydrocarbons, alcohols, ethers, ketones, alkylphenols, etc.

The aliphatic hypnotics provide the classical example of drugs which follow the simple geometrical increase of activity as homologous series are ascended. Three famous theories were postulated to account for the experimental facts: the Overton-Meyer theory relied upon the partition coefficients between media of administration and tissue lipoids; the Traube theory attempted to relate hypnotic activity to the lowering of surface tension at an air-water surface; and the Warburg theory relied upon the adsorption of aliphatic narcotics at cell surfaces. All three theories depended, it may be noted, upon a distribution of the drug between two phases, usually an aqueous or gaseous medium of administration and a non-aqueous cell membrane.

As has already been mentioned (p. 308), FERGUSON [4] pointed out that all the common physicochemical properties of homologous series, which depend upon a distribution between two phases, will increase or decrease geometrically as a series is ascended arithmetically, a result which follows from the constant increment of free energy for each additional methylene group, so that there is no way of deciding between the three theories of narcosis mentioned above. At the same time FERGUSON drew attention to the fact that any distribution between two phases, the aqueous or gaseous phase of the administered drug and the 'biophase' in which the drug has its distinctive effect, must involve, at equilibrium, equal chemical potentials in both phases.

The thermodynamic concept of chemical potential is rather remote from chemical pharmacology. FERGUSON equated it with the 'partial molal free

energy' of the drug, referred to some standard state. For ideal gases and ideal solutions the partial free energy F is given by the equation

$$F = F_0 + RT \ln C,$$

where F_0 is the partial molal free energy at some standard state, R the gas constant, T the absolute temperature and C the molal concentration (moles of drug per total moles of solution or gas). For non-ideal gases and dilute solutions, C must be replaced by thermodynamic activity a, which is often a complex function of C, so that the free energy equation becomes

$$F = F_0 + RT \ln a.$$

But the thermodynamic activities of drugs are rarely known and often difficult to estimate so that FERGUSON replaced a by the admittedly approximate values p_t/p_s or C_t/C_s, where p_t and C_t represent toxic or pharmacological partial pressures and concentrations respectively, in molar (not molal) terms, and p_s and C_s are saturated vapour pressures and concentrations respectively at the experimental temperature.

By this series of approximations, so typical of the physical chemist's procedure, FERGUSON was able to demonstrate that enormous ranges of toxic or narcotic activities involved quite small changes of the values for p_t/p_s and C_t/C_s; thus the equitoxic molar concentrations of methanol and *n*-octanol on *B. typhosus* were 10·8 and 0·0034 respectively (a 3000-fold range) but the C_t/C_s ratios were 0·33 and 0·88 respectively.

As homologous series are ascended the ratio C_s/C_t increases slowly and eventually a member is reached for which the ratio is approximately unity, i.e. the member will only achieve the standard effect being measured when it is administered as its saturated solution. Higher members with lower partial pressures or aqueous solubilities will consequently be inactive or only feebly active. FERGUSON's theoretical treatment therefore accounts for the fact that homologous series, which display a geometrical increase in activity as the series is ascended, reach a maximal activity for one member and higher members have little or no activity; this 'cut-off', as it is called in laboratory jargon, is not peculiar to biological systems but has been observed in purely physical systems such as the adsorption of a solute on solid surfaces, and it will always be observed when the solubility decreases more rapidly, as a series is ascended, than the property determining distribution of the solute between two phases.

It has already been mentioned that in series for which activities increase as $1:n:n^2:n^3\ldots$, the value of n is usually 2·5 to 3·3, but the ratio between the water solubilities of successive members usually lies between 4 and 4·5, so that the decline in solubility is bound to overtake the decrease in equiactive molar concentration. This explains why higher hydrocarbons, alcohols, etc., have no narcotic activity.

The position of the 'cut-off' in any series will depend upon the sensitivity of the organism or tissue under investigation, and it will be obvious that the

less sensitive the biological system is, the earlier in the series will the 'cut-off' appear, simply because lower sensitivity means higher concentrations of the drug for the achievement of the standard effect.

FERGUSON's theory therefore provides a rational explanation of the geometrical increase in potency for certain pharmacological effects of some homologous series; it also accounts for the abrupt decline in potency beyond one member of a homologous series and for the fact that this 'cut-off' does not occur at the same member in a given series for all biological systems. At the same time it does not throw any light upon the mechanism of action of homologous series, which follow this mathematically simple rule, except that their action depends upon the attainment of a critical concentration of the drug in some unspecified 'biophase'. It would be foolish to assume that all homologous series of drugs which follow the Ferguson pattern of behaviour act in the same way; all we can say is that whatever mechanism of action is involved, it is dependent upon a distribution between two phases, the gaseous or aqueous phases of administration and the 'biophase', whatever that may be, within which the drug has its effect; and upon the attainment of a critical concentration of the drug in the hypothetical biophase. Whether this critical concentration fills up the intermolecular spaces in cell membranes, as MULLINS [5] has suggested; whether it prevents the access of normal substrates to respiratory enzymes by a film of impenetrable material; whether it alters in some, at present unforeseen way, the physicochemical properties of cells; none of these possible mechanisms has been firmly established by experimental work, but what we can be certain about is that the pharmacological effect of any member of a homologous series which 'obeys' the Ferguson rule must depend primarily upon its physicochemical properties.

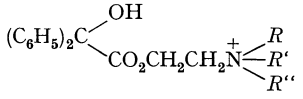
Compounds of this type might be expected to be easier to deal with, from a structure-action relationship point of view, than compounds, closely related in chemical structure, which show no simple relationships between potency and chain-length. Curiously enough, this is not true. We know more about the structure-action relationships of homologous drugs which display irregular and unexpected potencies than about series the potencies of which follow some simple mathematical formula. Probably this is because an unexpected and irregular change of potency, resulting from the addition of one methylene group to a drug molecule, gives us a better clue to the intimate nature of drug action than the regular increase in potency of the so-called biological depressants.

2.2 *Irregular Increase in Activity Within Series*

The commonest way in which pharmacological activities vary within homologous series is that activity increases irregularly as a series is ascended, reaches a maximal value for one (or occasionally two) members and then declines irregularly.

A typical example of this kind of effect is illustrated by the atropine-like properties of the benzilic esters of β-hydroxyethyl-alkyldimethyl- and -alkyl-diethylammonium salts [6, 7], the relative molar potencies of which are given in Table 1. It will be noticed that for the alkyldimethylammonium series

Table 1. *Atropine-like Properties of Homologous Benzilic Esters*



Nature of the N-alkyl groups			Relative molar potencies in terms of atropine = 100		
R	*R'*	*R''*	On the salivary gland (cat)	On blood pressure (cat)	On the eye (mouse)
CH_3	CH_3	H	11 ± 1·8	9·8	12·6
CH_3	CH_3	CH_3	196 ± 32	103	31
CH_3	CH_3	C_2H_5	258 ± 38	182	104
CH_3	CH_3	*n*-C_3H_7	147 ± 46	98	22
CH_3	CH_3	*n*-C_4H_9	75 ± 19	63	10·5
CH_3	CH_3	*n*-C_5H_{11}	71 ± 36	32	13
C_2H_5	C_2H_5	H	18 ± 4·6	21	6·3
C_2H_5	C_2H_5	CH_3	273 ± 49	239	64
C_2H_5	C_2H_5	C_2H_5	273 ± 44	190	83
C_2H_5	C_2H_5	*n*-C_3H_7	135 ± 30	78	22

maximal activity occurs for the ethyldimethyl member, whereas in the alkyl-diethylammonium series maximal activity occurs when the alkyl group is either methyl or ethyl. How are we to explain results of this kind?

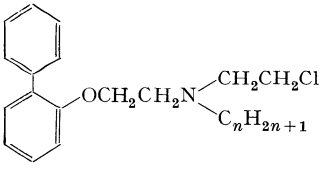
BARGER [8] suggested that the activity of any member of a homologous series depended upon the summation of two opposing effects, which he thought of as physical in nature. If one physical property changes as a series is ascended in a direction favourable to a particular pharmacological response, but a second physical property changes in an unfavourable direction, maximal activity must occur for the member which has an optimal combination of two opposing physical properties. In principle, BARGER's view has much to recommend it, but it is probably too simple. Maximal activity within a homologous series must depend ultimately upon an exceptionally favourable combination of physical properties, but there may well be more than two, and some of them, like stereochemical configuration, molecular conformation, etc., cannot be defined in terms of a stepwise increase or decrease, that can be plotted in terms of two co-ordinates.

What BARGER did not take into account was the unique properties of macromolecules (proteins, lipoproteins, nucleoproteins, etc.) upon which relatively small drug molecules are supposed to act. It may well be that one member of a homologous series has exceptional activity, not because of a nice balance of orthodox physicochemical properties, but because it fits in a unique way into a macromolecule involved in a complex biochemical situation.

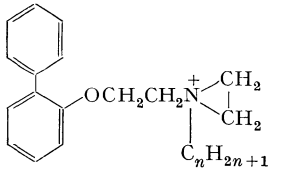
Ultimately drug action will, no doubt, be interpreted in physicochemical terms, but at the moment we cannot achieve this, because we know so little about the tissue constituents, call them receptors or what you will, upon which drug molecules act.

Other difficulties complicate our problem. Even within homologous series we cannot assume that every member acts in the same way; thus, although alkyltrimethylammonium salts, from alkyl = methyl to decyl, all produce neuromuscular block; the methyl to octyl members do so by depolarization of the motor endplate but the decyl member does so by competitive block (see 2.4 and 2.5 below).

When numerous members of a homologous series all have some similar pharmacological property, probably with maximal activity for one member, it is natural to assume that all members act, more or less efficiently, in the same way. Sometimes this assumption is reasonable, especially when we have some clue to the mechanism of action of the series. Two examples may be mentioned: (i) It is generally accepted that the anti-adrenaline β-chloroethylamines act as alkylating agents, via the ethylene immonium cations which they form more or less readily, so that in the series (I) studied by LOEW and MICETICH [9] the active molecular species is probably the cation (II). It was found that the



(I)

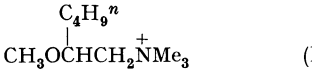


(II)

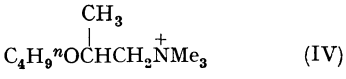
n-propyl to n-hexyl members were approximately equipotent but the methyl an ethyl members were less active. Several explanations of the inferior activity of the first two members could be suggested, but it would be fruitless to pursue the problem in the absence of physicochemical studies of the series. (ii) There is little doubt that trypanocidal arsenicals owe their curative properties to the arsenoxide group, so that it is surprising, in the polymethylene series p-OAs $C_6H_4(CH_2)_nCO_2H$, to find activity sharply dependent upon the length of the polymethylene chain, butarsen ($n = 3$) being much more active than its next lower and higher homologues [10].

The two examples mentioned above both involve, as we suppose, true chemical combination (i.e. covalent combination) with tissue constituents.

Where drugs form non-covalent combinations with receptors, due to London forces, hydrogen bonding, polar attraction, etc., we cannot be so certain that homologues are acting in the same way, although the ultimate pharmacological results are similar. Sometimes only one homologue fulfils the necessary conditions, higher or lower homologues being either inactive or antagonistic; thus QUASTEL *et al.* [11] found that succinic dehydrogenase was highly selective; it would dehydrogenate succinic acid, but was inhibited by both malonic and glutaric acids (the next lower and higher homologues respectively). Few enzymes are so selective among homologues as succinic dehydrogenase; their selectivity is more likely to be stereochemical. Among drugs a striking example is the failure of acetyl-β-methylcholine to produce a rise of arterial blood pressure after atropine [12]; even more remarkable was the failure of 3-keto-2-methylamyltrimethyl-ammonium ($CH_3CH_2COCHMeCH_2\overset{+}{N}Me_3$) to produce a pressor effect after atropine, although its next lower homologue (3-ketoamyltrimethyl-ammonium, ($CH_3CH_2COCH_2CH_2\overset{+}{N}Me_3$) had a purely pressor effect upon the cat's blood pressure even before the administration of atropine [13]. Since SIMONART [14] in a study of choline ethers noted that the *n*-butyl ether of choline had a purely pressor effect upon arterial blood pressure, whereas the methyl ether had a vasodilator effect, it is worth noting that of the isomeric ethers (III) and (IV)



(III)



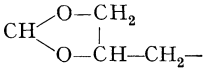
(IV)

the former (III) produced a striking pressor effect after atropine whereas the latter (IV) produced only a slight rise; on the other hand the latter (IV) had practically no vasodilator effect before atropine (compare choline butyl ether), whereas the former (III) had a vasodilator effect midway between those of the methyl and ethyl ethers of β-methylcholine. Consequently the conflicting effects of the methyl and butyl groups in choline alkyl ethers depend upon which of them is attached to the oxygen and which to the β-C-atom. This one example illustrates the difficulty of predicting the effect of extending an alkyl chain in drug molecules.

One regularity has been observed for the parasympathomimetic properties of cholinomimetic drugs and has been summarized in ING's empirical 5-atom rule [13, 15, 17]; this is illustrated in Table 2. At the same time it must be admitted that the 'nicotinic' properties of cholinomimetic drugs follow no such simple rule.

WHITTAKER *et al.* [16] examined the substrate specificity of alkyl esters of fatty acids for the different cholinesterases of the horse. For the erythrocyte enzyme (acetocholinesterase) the optimal acyl group was acetyl; for the plasma enzyme (butyrocholinesterase) it was butyryl. But for both enzymes, whatever

Table 2. *Parasympathomimetic Homologues,* $R\overset{+}{N}Me_3$

R	Most active when $n =$	Number of atoms in chain *R*
$C_nH_{2n+1}CO_2CH_2CH_2-$	1	5
$C_nH_{2n+1}NHCO_2CH_2CH_2-$	0	5
$C_nH_{2n+1}OCH_2CH_2-$	2	5
$C_nH_{2n+1}OCHMeCH_2-$	2	5
$C_nH_{2n+1}OCHEtCH_2-$	1	4
$C_nH_{2n+1}OCHPrCH_2-$	2	5
$C_nH_{2n+1}OCH_2-$	3	5
$C_nH_{2n+1}OCH_2CH_2CH_2-$	1	5
$CH_3CO_2(CH_2)_n-$	2	5
$C_nH_{2n+1}-$	4 and 5	4 and 5
C_nH_{2n+1} 	1	5
C_nH_{2n+1} 	1	5

the acyl group, the rate of hydrolysis depended on the alkyl group of the esterifying alcohol in the following way:

$$CH_3 < C_2H_5 < n\text{–}C_3H_7 < n\text{–}C_4H_9 < iso\text{–}C_4H_9 < (CH_3)_2CH \cdot CH_2CH_2 < (CH_3)_3C \cdot CH_2 \cdot CH_2.$$

Although the last three alkyl groups do not strictly belong to the same homologous series as the first four, this seems to be a convenient point to mention this important work, especially as the most active alkyl group approximates in configuration to the choline group of acetylcholine.

Polymethylene homologues with terminal basic groups provide many examples of the occurrence of maximal activity for one member. For example, polymethylene diamines $\{NH_2(CH_2)_nNH_2\}$ are oxidized by both amine oxidase and diamine oxidase [18], but the maximal rates for the two enzymes differ widely, as indicated below (the figures in bold type referring to homologues with maximal rates):

amine oxidase: no affinity up to $n=6$ and then

$$n=7=8=9<10<11<12<\mathbf{13}>14>15>16>18$$

diamine oxidase: $n=2<3<\mathbf{4}>5>6>7>8$

$n=9$ gives a compound which is not oxidized but acts as an inhibitor of the enzyme.

It seems clear that the specific requirements of diamine oxidase are more exacting than those of amine oxidase; if diamine oxidase requires the attachment of both amino groups, this is not surprising, since the distance between

the two amino groups will determine the optimal rate of oxidation. On the other hand, amine oxidase oxidizes many monoamines, so that a second terminal amino group might be expected to interfere with the enzyme unless it was sufficiently far away for the diamine to behave towards the enzyme as a monoamine; this is probably why diamines up to $n=6$ have no affinity for the enzyme and why an optimal rate occurs for so long a chain as $n=13$.

The polymethylene diamidines $\{NH{=}(NH_2)C{-}(CH_2)_n{-}C(NH_2){=}NH\}$ have been studied on at least five pharmacological preparations, although unfortunately not always with complete series.

As inhibitors of the oxidation of tyramine by amine oxidase the order of efficiency was [19]:

$$n = 7<8<9<10<11<\mathbf{12}>13>14>16.$$

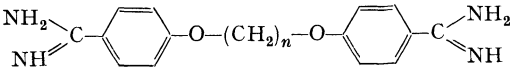
These results are interesting because the most potent inhibitor ($n=12$) contains a polymethylene chain only one C-atom less than the optimal polymethylene diamine substrate ($n=13$).

The series $n=8$–14 and 16 were tested as trypanocides on *T. Rhodesiense in vitro* by KING, LOURIE, and YORKE [20]. High activity was confined to the group for which $n=10$–14, the member with $n=11$ being the most active, but all members of the C_{10}–C_{14} group were curative in mouse infections.

The same series was tested for bactericidal activity *in vitro* by FULLER [21], who found no obvious peak for either gram-positive or gram-negative organisms. As might be expected, activity increased slowly throughout the series but was decreased, especially for the higher members, in the presence of serum proteins.

BROOM [22] found that the members with $n=3$, 5, and 11 were hyperglycemic, whereas the members with $n=7$, 8, and 10 were hypoglycemic, the C_8 member being about twice as active as the other two. DAWES [23] studied the C_7, C_{10}, C_{12}, C_{14}, and C_{16} members as potentiators of adrenaline after intraportal injection and found that the C_{14} compound was the most active.

Thus for the polymethylene diamidines maximal activities occur at different chain-lengths for five different biological activities. Probably the most interesting results are those on *T. Rhodesiense* (max. for $n=11$) since in the series (V) the most effective compounds in African trypanosomiasis are propa-



(V)

midine ($n=3$) and pentamidine ($n=5$) [24]. If we take the benzene ring as roughly equivalent in length to trimethylene and the oxygen atom as only slightly larger than the carbon atom, it will be seen that propamidine ($n=3$) has approximately the same distance between the amidine groups as 1,11-undecane diamidine, which was the most active against *T. Rhodesiense* of the polymethylene diamidines; this may be a chance result, since it is difficult to

relate *in vitro* experiments to curative properties in animals. Actually, pentamidine ($n=5$) has proved to be the most effective drug of the diamidine series against early infections of *T. gambiensa* and *T. rhodesiensa*.

Another series of great interest is the ganglion-blocking polymethylene bis-quaternaries, $R_3\overset{+}{N}(CH_2)_n\overset{+}{N}R_3$; among compounds of this type activity is restricted to a narrow range of n (4-6) [25], but what is interesting is that within this narrow range optimal blocking activity is dependent on the alkylation of the N-atoms, as shown in Table 3 in which WIEN's results [26] have

Table 3.
Relative Molar Potency (Hexamethonium = 100) on the Superior Cervical Ganglion (Cat)

$R_3\overset{+}{N}$–	$R_3\overset{+}{N}$–$(CH_2)_n$–$\overset{+}{N}R_3$				$R_3\overset{+}{N}$–C$_6$H$_4$–$(CH_2)_n\overset{+}{N}R_3$			
	$n=4$	$n=5$	$n=6$	$n=7$	$n=1$	$n=2$	$n=3$	$n=4$
$Me_3\overset{+}{N}$–	1	63	100	12	0·6	395	34	7
$Me_2Et\overset{+}{N}$–	10	156	162	11	–	459	72	15
$MeEt_2\overset{+}{N}$–	108	140	87	18	–	267	23	8
$Et_3\overset{+}{N}$–	6	8	6	3	–	3	6	8

been converted into molar terms. It will be noticed that as N-methyl groups are successively replaced by ethyl maximal activity shifts from the hexamethylene to the pentamethylene chain; also that when both quaternary groups are $Et_3\overset{+}{N}$– the length of the polymethylene chain ($n=4$–7) scarcely affects the feeble activity of these compounds.

In the phenylpolymethylene series [27] p-$R_3\overset{+}{N}C_6H_4(CH_2)_n\overset{+}{N}R_3$ (Table 3) high ganglion blocking activity is confined to compounds in which $n=2$, whether the terminal groups are Me_3N–, Me_2EtN– or $MeEt_2N$–; this difference from the polymethylene series is probably due to the rigid structure of the phenyl group which prevents such a gradual change of chain length (or rather interionic distance) as can occur in the polymethylene series. Nevertheless GILL's calculations [28] of interionic distance in both series, calculations which took into account both the restricted rotation about single C–C bonds in the polymethylene chain and the mutual repulsion of the two cationic groups, clearly show that ganglion blocking activity among bis-quaternaries of these two series is prominent only in compounds with an interionic distance of 6-7·8 Å. GILL's calculations referred only to bis-trimethylammonium compounds, but it is obvious that the replacement of one or two methyl groups at each end of the molecule by ethyl will tend to extend the interionic distance slightly.

The azamethonium series $R_3\overset{+}{N}CH_2CH_2NR \cdot CH_2CH_2\overset{+}{N}R_3$ in which one or more of the terminal R groups has been changed has already been reviewed in an earlier volume of this series [29]. It may be recalled, however, that the most highly active members, whether the central N-atom is secondary (NH) or tertiary (NMe) are those in which the terminal groups are $-NMe_2Et$ or $-MeNC_4H_8$.

The high activity of the bis-N-methylpyrrolidinium compounds in both the methonium [30] and the azamethonium [29] series is interesting; in both series the replacement of the pyrrolidine by the piperidine group reduces activity, an example of a *cyclo*polymethylene series $\{(CH_2)>\}N$ mentioned in 1.1. Also in both bis-pyrrolidine series maximal activity occurs for a five atom chain $\{(CH_2)_5$ or $(CH_2)_2NR(CH_2)_2\}$, a result which agrees with maximal activity occurring in the polymethylene series for a 5-atom chain when the terminal groups are $-NEt_2Me$ (Table 3).

It seems, therefore, that in both the methonium and azamethonium series, the results are reasonably consistent. This does not mean that we can explain the results, but only that, in these two series, structure-action relationships do not present us with difficult inconsistencies.

The neuromuscular blocking activities of bis-quaternary ammonium salts also provide interesting evidence about polymethylene homologues. BOVET *et al.* were the first workers to show that maximal blocking activity occurred when the two quaternary groups were separated by a 10-atom chain, but it was BARLOW and ING [31], and PATON and ZAIMIS [25], who first studied the simplest homologous series, viz. the polymethylene bis-trimethylammonium series. They found that decamethonium, $Me_3\overset{+}{N}(CH_2)_{10}\overset{+}{N}Me_3$, was the most effective member, and later BOVET *et al.* [32] showed that among the choline esters of α-ω dibasic acids, $Me_3\overset{+}{N}CH_2CH_2OCO(CH_2)_nCO \cdot OCH_2CH_2\overset{+}{N}Me_3$, it was the succinate ester ($n = 2$, chain length = 10) that had the highest neuromuscular blocking activity.

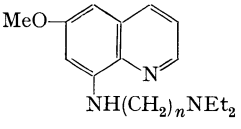
The polymethylene bis-trimethylammonium series is of particular interest because these simple compounds display so many biological properties, and optimal activities occur for different effects at different chain lengths. Thus for ganglionic block maximal activity occurs for a 5 or 6 C-chain; for neuromuscular block at the member with a 10 C-chain; for contracture of the frog's rectus abdominis, muscarine-like activity on guinea pig ileum, and anti-acetocholinesterase activity, less well defined maxima occur around the 12 C-chain [25]. The last effects resemble closely those of mono-quaternary ammonium salts like $Me_4\overset{+}{N}$, whereas the ganglionic and neuromuscular blocking effects, with their sharp dependence upon the length of the polymethylene chain, would seem to be determined by an attachment of both cationic groups to anionic centres in both types of synapse. An enzymic analogy has already been mentioned (p. 321). GILL's calculations of interionic distance in the two series: $Me_3\overset{+}{N}(CH_2)_n\overset{+}{N}Me_3$ and $Me_3\overset{+}{N}C_6H_e(CH_2)_n\overset{+}{N}Me_3$ have also been discussed (p. 323). It would seem, therefore, that in the series $Me_3\overset{+}{N}(CH_2)_n\overset{+}{N}Me_3$ some pharamco-

logical properties are sharply dependent upon the value of n, whereas others are less dependent, but owe their effects mainly to the strongly basic character of the molecule, and can be imitated, qualitatively and quantitatively, by simple mono-quaternary ammonium cations.

2.3 *Alternating Activities Within Series*

It has already been mentioned (section 1.2) that some polymethylene series display alternating physical properties, but that the latter are usually restricted to properties, like melting-points and aqueous solubilities, that depend upon an equilibrium between the solid crystalline substance and its liquid phase or solution; in other words, upon the different methods of packing of alternate members of homologous series in the crystal. It is therefore surprising to find that some homologous series display alternating pharmacological activities.

One of the first examples to be recorded was the observation by MAGIDSON *et al.* [33] that the chemotherapeutic indices (MTD/MCD) of 6-methoxy-8-aminoquinoline antimalarials (VI) were consistently higher when n was an odd number than when n was an even number (range $n=2$ to 7). The chemotherapeutic index (MTD/MCD) is too complex a function to throw light upon this curious alternation.



(VI)

A simpler example, and one of great interest because it occurs in an alkane series, was discovered by BUTTLE *et al.* [34] during their investigation of the antimalarial activities of n-alkyl ethers of apoquinine and of dihydrocupreine. These authors used *P. relictum* injections of canaries, and expressed their results as 'quinine ratios' $(z-x)/(y-x)$, where x is the mean delay in days for the appearance of parasites in the blood of untreated birds, y the mean delay for quinine and z the mean delay for the alkyl ether being tested. In the series of alkyl ethers of apoquinine a striking alternation occurred for the n-amyl to n-undecyl member, ethers with an even number of C-atoms in the alkyl group having consistently higher quinine ratios than ethers with an odd number of C-atoms in the alkyl group. The alkyl ethers of dihydrocupreine showed no such alternation, but curiously alkyl ethers with an odd number of C-atoms from 1 to 11 displayed some alternation of quinine ratios.

It is worth noting that an entirely different alkane series, viz. the diphenyleneoxy ethyl-alkyl-β-chlorethylamines (I) investigated by LOEW and MICETICH [9] displayed a definite alternation of toxicities (LD_{50} values) in mice for the series C_nH_{2n+1} = methyl to n-hexyl, although the anti-adrenaline properties of this series showed no alternation (see 2.2).

A recent example of some interest was encountered by RAISON and STANDEN [35] in a series of 4,4′-dimethylamino-diphenoxy-alkanes,

$$4\text{-}Me_2NC_6H_4O(CH_2)_nOC_6H_4NMe_2(4'),$$

which were being tested for schistosomocidal activity. Over the range $n=2$ to 10 alternation of activities occurred. Moreover the same compounds showed alternation of melting points and aqueous solubilities. This alternation of physical properties was retained when the 4,4′-dimethylamino groups were replaced by amino or methylamino groups, whereas replacement of the terminal tertiary amino groups by primary or secondary amino groups abolished alternation of schistosomocidal activities.

In considering alternation in pharmacological properties it must be remembered that it occurs in alkane series as well as in polymethylene series, so that the most likely explanation of the phenomenon will depend upon the rates of metabolism and excretion of successive members. The observation of RAISON and STANDEN that alternation only occurred for series of bis-tertiary bases is readily explained by the fact that metabolic pathways are open to bis-primary of bis-secondary bases (e.g. acetylation) which are not open to bis-tertiary bases.

That the metabolic end product of polymethylene series is often dependent on the value of n is well known. The classical work of KNOOP and of DAKIN on ω-phenyl fatty acids, $Ph(CH_2)_nCO_2H$, proved that when n was an odd number the acid was excreted as phenylacetic acid or a conjugate of it, but that when n was an even number the acid was excreted as a conjugate of benzoic acid. Similarly ω-fluoro fatty acids $F(CH_2)_nCO_2H$ in which n is an odd number are much more toxic than those in which n is an even number [36], presumably because the former can be degraded by β-oxidation in the animal to fluoro-acetic acid.

Consequently it seems likely that when alternation of pharmacological properties occurs this is due to alternating rates of metabolism and excretion, and not to some peculiar properties of receptors in cells.

2.4 *Series with Diverse Actions*

One of the most intriguing examples of series of this kind is the N-alkyl noradrenaline series. In their classical work on sympathomimetic amines BARGER and DALE [37] noticed that noradrenaline was more active than adrenaline in producing a rise of arterial blood pressure in the cat but less active than adrenaline in relaxing plain muscle, such as uterine muscle, although the only chemical difference between the two substances is that noradrenaline has a terminal NH_2-group whereas adrenaline has a terminal $NHCH_3$-group. At the time (1910) noradrenaline was not known to be a naturally occurring substance in the animal body so that its greater activity on arterial blood pressure than the natural hormone adrenaline was strange.

When acetylcholine was clearly established as the chemical transmitter at parasympathetic nerve endings it was natural to conclude that adrenaline was the transmitter at sympathetic nerve endings, but the work of CANNON and ROSENBLÄTH suggested that there were two sympathetic transmitters: Sympathin E (excitatory) and Sympathin I (inhibitory). The discovery that noradrenaline was the main transmitter at many sympathetic nerve endings resolved the main difficulty, but the problem of why noradrenaline and adrenaline differed quantitatively in their excitatory and inhibitory effects remained. It was clear that the two substances played different roles in the animal economy, and AHLQUIST [38] suggested that there might be two different types of receptor, which he called α- and β-receptors, the former triggering stimulant responses and the latter triggering relaxant responses. The multiplication of hypothetical receptors has obvious disadvantages, but it must be remembered that there were no such discriminating antagonists at adrenergic synapses as we have for cholinergic synapses; if it were not for drugs like atropine, hexamethonium and tubocurarine we should no doubt be talking of α-, β-, and γ-receptors for acetylcholine. It is in fact interesting to note that just as adrenaline has both Sympathin E and I properties so acetylcholine has both muscarine-like and nicotine-like properties. Similarly, just as the balance between the two types of activity associated with acetylcholine may be altered by the addition of one or more methylene groups (e.g. acetyl-β-methyl choline is purely muscarinic and propionylcholine is mainly nicotinic) so the addition or subtraction of a methylene group to or from the adrenaline molecule alters the pattern of activity, either to that of Sympathin E or to that of Sympathin I.

In general alkylation of noradrenaline reduces the hypertensive activity of the molecule in the order $-NH_2$, –NHMe, –NHEt, $NHPr^n$ and hypotensive effects occur when the terminal group is $-NHPr^{iso}$ or –NHBu (where the butyl group is normal, secondary or tertiary); branching of the N-Pr and N-Bu groups increases the hypotensive effect.

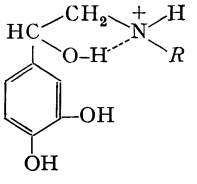
Compounds of the series which are hypotensive are also characterized by other β-effects, such as relaxation of plain muscle (e.g. bronchial, uterine and intestinal), the glycogenolytic effect and the corticostimulating action.

The transition between AHLQUIST's α- and β-effects is not sudden; adrenaline and to a lesser extent N-ethyl noradrenaline share both. PRATESI *et al.* [39] have attempted to explain the transition as the N-alkyl noradrenaline series is ascended by a study of the physicochemical properties of the series with particular attention to the effects of N-alkylation upon the basic properties of the N-atom and the hydrogen-bond between the side-chain alcoholic (OH) group and the basic (–NHR) group. In these studies he used, for the inductive effects of different alkyl groups, the polar constants (σ) defined by TAFT [40], the rest of the molecule $\{(HO)_2C_6H_3CHOH \cdot CH_2–\}$ being assigned an arbitrary constant (C) for all members of the series.

By plotting the experimental values of pK_1 against the values of $\Sigma\sigma$ (total polar constant, proportional to the global inductive effect of the N-alkyl group) he obtained two linear relationships: one for n-alkyl groups and another for

branched chain alkyl groups like *iso*propyl, *iso*-, *sec.*-, and *tert.*-butyl; and these linear relationships had different slopes.

Beside the effect of pK_1 values we have also to take into account the effect of the terminal alkyl group upon hydrogen bonding of the type illustrated



(VII)

(VII), remembering that the tendency to form hydrogen bonds decreases as the positive inductive effect of the alkyl group increases.

At physiological pH values adrenaline and most N-alkyl-noradrenaline derivatives will exist mainly as cations, so that we can foresee two types of interaction with receptors: ionic interaction with an anionic site, and hydrogen bonding with a different site. Both types of union may occur for any given molecule, but one may be more important than the other, and the overall effect may depend upon the predominant type of union. Thus noradrenaline should be exceptionally favourable as regards hydrogen bonding, whereas N-alkyl derivatives of it, and especially branched chain alkyl derivatives like the *iso*-propyl and *tert.*-butyl members, should offer less favourable conditions for hydrogen bonding.

Adrenaline and ethyl-noradrenaline, in PRATESI's view, form a transition series, both attracted to α- and β-receptors, whereas higher homologues, and especially compounds with a branched N-alkyl group, appear to be attracted mainly to β-receptors. In this connexion it is interesting to note that, so far as data are available, the N-alkyl derivatives of noradrenaline which act primarily on β-receptors appear to be just as stereo-specific as noradrenaline or adrenaline, so that it is probable that they form specific drug-β-receptor complexes.

2.5 *Partial Agonists*

A special case of homologous drugs with diverse actions is that of series in which higher members not only show declining activities, as the series is ascended, but also increasing ability to antagonize the effects of lower members. These higher members, which possess both stimulant and antagonistic properties, are called 'partial agonists'.

It is common knowledge that many drugs have a so-called diphasic action, stimulating some physiological mechanism in small doses but depressing it in larger doses (e.g. nicotine), or even producing stimulation followed by depression at any given dose level (e.g. decamethonium). It might well be asked, therefore, why there is any need for the term 'partial agonist'. STEPHENSON [41] de-

fined partial agonists in terms of his theory of drug action, which retained CLARK's idea of the affinity of a drug for a particular type of receptor but introduced the idea of 'efficacy'; by efficacy he meant the capacity of a drug to produce some standard response in terms of the proportion of the available receptors occupied. A drug with low efficacy would need to occupy more receptors than a drug with high efficacy, in order for both to produce the same response.

It would seem better to define partial agonists in terms of the experimental facts, rather than in terms of a particular theory of drug action. I propose to define partial agonists as drugs *'which have a stimulant action but will reduce the effect of another, usually more active drug, when both are present together'*.

The simplest, and probably the best investigated, series that includes partial agonists is that of the *n*-alkyltrimethylammonium salts, in which the effective molecular species is the cation $C_nH_{2n+1}\overset{+}{N}Me_3$. This series has been investigated on various isolated preparations and in different ways be numerous authors. It will be convenient to consider the effects of members of the series on different types of tissues separately; indeed, this is the only rational way of dealing with structure-action relationships among a group of chemically related drugs.

2.51 Effects on Plain Muscle

STEPHENSON [41] found that there was no simple relation between the value of *n* in cations $C_nH_{2n+1}\overset{+}{N}Me_3$ and their stimulant effect upon guinea pig ileum. For the first six members of the series the concentrations ($M \times 10^{-6}$) producing 50 per cent of the maximal contraction were 43·5, 52·0, 195, 1·33, $0{\cdot}582 \pm 0{\cdot}092$, and 2·48 respectively; i.e. activity decreased from $n=1$ to $n=3$, then increased sharply to a maximum for $n=5$, and decreased for $n=6$. That maximal activity should occur for the *n*-amyl member is in agreement with ING's '5-atom rule' for muscarinic quaternary salts, and is in accord with RAVENTÓS's results [42] with the same series on frog auricles.

Members with more than six C-atoms in the *n*-alkyl group were incapable of producing maximal contraction at concentrations as high as $M \times 10^{-4}$, and it is clear from STEPHENSON's results that the heptyl, octyl and nonyl members at this high concentration were producing their maximal effects, viz. about 40–60 per cent of that of the hexyl member. The decyl member produced less than 20 per cent of the maximal contraction at $M \times 10^{-5}$ and smaller contractions at higher concentrations. Moreover STEPHENSON showed that the octyl member, at a concentration ($4 \times 10^{-4} M$), which produced a contraction about one-third of that of the butyl member at $1{\cdot}6 \times 10^{-5} M$, could, when both drugs were administered together at these respective concentrations, reduce the response of the guinea pig ileum to little more than that produced by the octyl member alone. Even increasing the concentration of the butyl member four times ($6{\cdot}4 \times 10^{-5} M$) did not restore its effect to more than 85 per cent of its original value in the absence of the octyl member. It may be noted that hexa-

methonium (100 mg/l) was added to the Tyrode solution in all these experiments in order to eliminate effects on ganglion cells, and a small amount of mepyramine (10 μg/l) in order to block the effect of any histamine liberated by the hexamethonium.

That the octyl member could act as a true atropine-like compound was demonstrated many years earlier by CLARK and RAVENTÓS [43], who measured what SCHILD [44] later called its pA_{10}, against acetylcholine and tetramethyl-ammonium and found closely similar values on frog auricle and rat intestine although tetramethyl-ammonium has about one thousandth of the activity of acetylcholine at parasympathetic nerve endings.

LING examined a longer series ($n = 1$–12) on both guinea pig ileum and rabbit bladder (unpublished results) but his results differ only in minor details from those of STEPHENSON. He did not find so sharp a maximum for the *n*-amyl member, but the *n*-butyl and *n*-amyl members were the most active members of the series. On the rabbit bladder his results clearly indicate that the *n*-heptyl member was, so to speak, the turning point in the series, i.e. that it was a partial agonist, whereas the *n*-decyl to *n*-dodecyl members had no stimulant activity.

STEPHENSON [41] exposed guinea pig ileum to various concentrations of *n*-alkyltrimethylammonium salts for 15 sec. PATON [45] found that if this tissue was in contact with members of this series for 60–90 sec the immediate contraction was followed by a rapid decline to an equilibrium value, a phenomenon to which he refers as 'fade'. In Figure 14b of his paper it can be seen that the immediate responses of the *n*-heptyl to *n*-undecyl members decreases as the series is ascended (he used a fixed dose of 200 μg per 20 ml bath instead of equimolar doses) and that the equilibrium values after 'fade' also decrease but less steeply. He reports that the 'fade' with the undecyl and dodecyl (and sometimes with the decyl member) was so complete that at equilibrium these members were devoid of stimulant action. His results are therefore not in conflict with those of STEPHENSON, but give additional information about the behaviour of higher members of the series. At the same time his interpretation of the action of this homologous series was different from that of STEPHENSON [41] and of ARIËNS [46] (see below).

PATON attributes excitation, not to the occupation of receptors (as CLARK, STEPHENSON, and ARIËNS did) but to the *process* of occupation, i.e. to the *rate* of occupation. It is an essential feature of PATON's theory that occupied receptors are immobilized, or as he writes 'receptor occupation necessarily and always implies the existence of antagonism'. Consequently the dissociation constant of the drug-receptor complex is decisive in distinguishing between agonists, partial agonists and antagonists: high values of the dissociation constant characterize pure agonists, low values pure antagonists and intermediate values partial agonists. One of the advantages of PATON's theory is that it implies no sharp distinction between agonists and antagonists, for even antagonists may display vestigial stimulation before block, so that partial agonists are necessarily implied by his theory. Moreover his theory expects and accounts for the phenomenon of 'fade'.

It is not within the province of this article to consider theories of drug action in detail, but it must be said that STEPHENSON's concept of 'efficacy' and ARIËN's idea of 'intrinsic activity' (see below) can be given no chemical meaning at present, whereas PATON's rate theory might be given a chemical meaning if rate constants could be measured at different temperatures.

Whereas STEPHENSON and PATON both used individual doses, ARIËNS *et al.* [46] used cumulative doses. VAN ROSSUM [47] has attempted to justify the cumulative dose method on the grounds that, given a stable drug and no fading of the tissue response, dose-response curves can be more easily and quickly obtained by a stepwise increase of the drug in the bath fluid surrounding an isolated tissue. This may be true if all we are interested in is the dose required for a maximal response of the tissue, but several criticisms of his method can be made: (i) successive doses of drugs without wash-out is the ideal method of reducing the sensitivity of the isolated tissue; (ii) the cumulative doses required for a 50 per cent response of the tissue differ by a factor of about 3 for the two methods. This is important because it is on the 50 per cent response that ARIËNS and VAN ROSSUM base their estimate of the affinity constant.

Like STEPHENSON, but unlike PATON, ARIËNS and his group distinguish between the affinity of a drug for hypothetical receptors and 'intrinsic activity', the latter concept being comparable with STEPHENSON's 'efficacy'.

Whereas affinity can be given a real chemical meaning in terms of the Mass Action law, the terms 'efficacy' and 'intrinisc activity' can only be interpreted in terms of the Michaelis theory of enzymic activity, the theory upon which ARIËNS based his ideas on drug action. But MICHAELIS worked with enzyme preparations *in vitro*, whereas the pharmacologist is primarily interested in *organized* tissues, whether *in vivo* or *in vitro*. A piece of plain muscle *in vitro* is obviously a much more complicated system than a homogenate containing various enzymes. In short, the ideas of enzymologists cannot be applied directly to isolated tissues because the latter have a *structure* which is essential to their behaviour. Consequently the somewhat less ambitious theory of PATON seems to the author more in line with pharmacological facts than the theories of STEPHENSON and ARIËNS. PATON's theory makes no assumptions about the nature of the hypothetical receptors upon which drugs act, and his empirical equations do not assume that they behave like enzymes.

2.52 NEUROMUSCULAR BLOCKING ACTIVITY

CRUM BROWN and FRASER [48] were the first to observe the paralysis of voluntary muscle by tetramethylammonium and KULZ [49] was the first to study the series $C_nH_{2n+1}\overset{+}{N}Me_3$ quantitatively; he attempted to estimate the minimum concentrations of members C_1 to C_8 required to paralyse completely the isolated sciatic-gastrocnemius preparation of the frog. He found that the *n*-propyl member was the least active and higher members (up to C_8) were approximately equal in activity to tetramethylammonium. ING and WRIGHT

[50] used the thin sartorius muscle of *Rana esculenta* (in order to minimize diffusion factors) and plotted the response to indirect stimulation against time, when the preparation was exposed to equi-millimolar solutions. They found that the ethyl member was the least active, but the C_4 to C_8 members all had about the same activity as tetramethylammonium at 1·0–0·5 mM/l. The C_{12} member was much less active, but this is probably due to its surface active properties and to micelle formation even at the relatively low concentrations used.

Since alkyltrimethylammonium salts are relatively weak neuromuscular blocking agents, compared with tubocurarine and synthetic bisquaternary salts, they have been neglected until recently when PATON and WAUD [51] re-investigated the series on the gracilis muscle of the cat's hindleg; they recorded the potential of an electrode drawn along the surface of a muscle fascicle against distance traversed, each trace being taken 2 min after the injection of 5 μmoles of a member of the series. They found that the depolarization peak decreased steadily from C_6 to C_{10}. Also, that given in a 90 per cent blocking dose, the hexyl, heptyl and octyl members produced depolarizations comparable with that of succinylcholine, whereas with the decyl member the depolarization was trivial or hardly detectable, the depolarization produced by the nonyl member being intermediate between the octyl and decyl members. Consequently in this series and on the cat's gracilis muscle the addition of two methylene groups to an alkyl chain already 8 carbon atoms long is enough to transform the neuromuscular blocking action from a depolarizing to a competitive one. If we regard depolarization as an activation process (which seems reasonable) only the nonyl member is a partial agonist, the decyl member being a pure antagonist. PATON and WAUD suggest that this abrupt transition is to be expected if we suppose that the drug-receptor dissociation constant falls by a factor of about 2·5 for each additional methylene group, since occupancy of receptors will be inversely related to dissociation. The tenacity with which a cation is held at an anionic receptor will obviously depend upon the length of an N-alkyl group, since if we start with a member whose occupancy is around 15 per cent, the addition of two methylene groups to the N-alkyl group will increase occupancy by $15 \times 2{\cdot}5^2$ per cent, that is over 90 per cent. As PATON and WAUD say 'the theoretical uncertainty lies, not in accounting for the abrupt transition, but rather in estimating at what chain length it will occur'.

It is clear that the transition between depolarization and competitive block of striated muscle will occur at different points within a homologous series according to the particular tissue upon which a series is tested. This is exemplified by the fact that decamethonium can act either as a depolarizing agent or as a competitor of acetylcholine at neuromuscular junctions in different species or even at different anatomical sites in the same animal.

It cannot be too strongly urged that structure-action relationships only have meaning in terms of some particular tissue. Much of the confusion about structure-action relationships arises because the chemist and the pharmacologist both attempt to elucidate all-embracing simple relationships from experimental results on a variety of different tissues. Once this mistaken ambition is

given up, the possibility of rationalizing struture-action relationships in reference to a particular tissue becomes a worthwhile project.

2.6 *Cyclopolymethylene Series*

Few examples of these types of drugs have been investigated systematically; probably the best examples are anti-hypertensive drugs of the general type $(CH_2)_n{>}NCH_2CH_2X$ where X is an amidoxine $\left(-N\begin{matrix}=NOH\\ \diagdown NH_2\end{matrix}\right)$, guanidine $\left(-NHC\begin{matrix}=NH\\ \diagdown NH_2\end{matrix}\right)$, or amidine $\left(-C\begin{matrix}=NH\\ \diagdown NH_2\end{matrix}\right)$ group. The activity of compounds of these three types appears to depend upon the value of n, or in other words upon the size of the terminal ring system. The subject has been reviewed by SCHLITTLER *et al.* [29], but unfortunately they give only qualitative data. However, the general situation is reasonably clear. In compounds of the general type (VIII)

$$(CH_2)_n{>}NCH_2CH_2X, \qquad \text{(VIII)}$$

where X is an amidoxine group $\left(N\begin{matrix}=NOH\\ \diagdown NH_2\end{matrix}\right)$, or a guanidine group $\left(NHC\begin{matrix}=NH_2\\ \diagdown NH_2\end{matrix}\right)$ high antihypertensive activity is confined to compounds in which $n=6$–8. In the amidine series $\left(X{=}C\begin{matrix}=NH\\ \diagdown NH_2\end{matrix}\right)$ high activity is confined (so far as compounds were tested) to compounds in which n was 6 or 7. It appears that the most active member of the series is guanethidine ($n=7$; $X=NH\cdot C(:NH)NH_2$) but one cannot help regretting that the Swiss workers give no quantitative information about these ring homologues; nor do they provide evidence that the less active members act in the same way as guanethidine. The mechanism of action of guanethidine is still obscure. It certainly suppresses, like bretylium, the action of postganglionic sympathetic fibres, but it also has ganglion blocking activity, and has mild local anaesthetic activity, but as it does not produce cholinergic block it is unlikely that the adrenergic block that it produces is due to its local anaesthetic properties or to its ganglion blocking action. The intimate mechanism of its action on sympathetic nerves remains to be discovered. In particular why high activity should be dependent upon the number of methylene groups in the heterocyclic ring is entirely unknown.

3. Hybrid Series

The most interesting hybrid series are those in which methyl is successively replaced by ethyl in quaternary ammonium salts. The classical example is at the same time the simplest: at the neuromuscular junction, blocking activity

decreases steeply for each successive replacement of methyl by ethyl in tetramethylammonium, so that we can write:

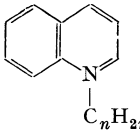
$$Me_4\overset{+}{N} > Me_3\overset{+}{N}Et > Me_2\overset{+}{N}Et_2 \gg Me\overset{+}{N}Et_3 \simeq \overset{+}{N}Et_4$$

tetraethylammonium being almost devoid of neuromuscular blocking activity. Indeed it has been shown to be an anticurare agent.

It might seem that increase in the size of the cation was responsible for this result, if it were not for the fact that tetraethylarsonium has about the same activity as tetramethylammonium. Indeed in the series:

$$R_4\overset{+}{N},\ R_4\overset{+}{P},\ R_4\overset{+}{As}$$

tetramethylarsonium is almost as inactive as tetraethylammonium and tetramethylphosphonium is less active than tetraethylphosphonium. Similarly in the alkylquinolinium series (IX):



(IX)

the order of neuromuscular blocking activity is

$$R = Me < Et < Pr = Bu = Octyl.$$

These results [50] are extremely difficult to understand, but they are not exceptional; for example, WIEN *et al.* [26] observed that in the transition series:

$$Me_4\overset{+}{N},\ Me_3\overset{+}{N}Et,\ Me_2\overset{+}{N}Et_2,\ Me\overset{+}{N}Et_3,\ \overset{+}{N}Et_4;$$

the first three members produced stimulation of the cat's superior cervical ganglion (in decreasing order of activity) followed by block, whereas the last two members produced block only. On the other hand in the methonium series the bis-dimethylethyl and -methyldiethyl members were the most potent ganglion-blocking agents (see Table 3). In these series the length of the polymethylene chain is also a determining factor: for $n=4$ terminal $-NMeEt_2$ groups are best, for $n=5$ or 6 terminal groups $-NMe_2Et$ are best. For the series $p\text{-}R_3\overset{+}{N}C_6H_4CH_2CH_2\overset{+}{N}R_3$ the best terminal groups are $-NMe_2Et$.

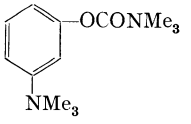
The occurrence of $-NMe_2Et$ as the most efficient terminal group in several classes of drugs that block acetylcholine is not unusual. For example it occurs for the mydriatic activities of benzilic esters and N-dibutylcarbamic esters of $HOCH_2CH_2\overset{+}{N}R_3$.

A particularly interesting example is provided by the anticholinesterase activities *in vitro* of compounds in which the N-methyl groups of neostigmine

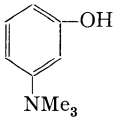
Table 4. *Hybrid Homologues of Neostigmine*

Compound	Anticurare Activity	Anticholinesterase Activity	
		pI_{50} values	
	pD_{20}	Acetocholinesterase	Butyrocholinesterase
Neostigmine (NMe_3)	7·60	7·4	7·2
Ro-1-3392 (NMe_2Et)	8·19	8·0	7·3
Ro-1-3393 ($NMeEt_2$)	8·57	8·2	8·0
S-208 (NEt_3)	6·59	7·2	7·4

(X) are successively replaced by ethyl groups. Reference to Table 4 will reveal that the most efficient quaternary ammonium group in these *in vitro* experiments was $-\overset{+}{N}MeEt_2$ (Ro-1-3393). Anticurare studies with the same series on the



(X)



(XI)

rat phrenic nerve-diaphragm preparation showed that Ro-1-3393 was also the most active, being 1·7 times as potent as neostigmine. Similarly in the phenolic compounds of type (XI) the most effective anticurare agent contained the $NMeEt_2$ group. However, it seems unlikely that the anticurare action of agents of type (XI) is due solely to anticholinesterase activity; their potentiation of muscle twitches would appear to be partly due to depolarization of the motor endplate (see STEMPEL and AESCHLIMANN [52]).

All the examples mentioned above must be considered in the light of the results of successive replacements of N-methyl groups by ethyl in acetylcholine, the natural chemical transmitter of motor nerves, preganglionic autonomic nerves and postganglionic parasympathomimetic nerves. HOLTON and ING [53] studied the hybrid series:

$$R\overset{+}{N}Me_3,\ R\overset{+}{N}Me_2Et,\ R\overset{+}{N}MeEt_2,\ R\overset{+}{N}Et_3,$$

where $R = CH_3CO_2CH_2CH_2-$ and their results are summarized in Table 5. It will be seen that replacement of one methyl group by ethyl reduced typical activities to between a half and a fifth of those of acetylcholine, whereas replacement of two or three methyl group by ethyl reduced all the activities drastically. It was concluded that at least two N-methyl groups were necessary for high acetylcholine-like activities. At the same time all members of the hybrid series were hydrolysed by acetocholinesterase at about the same rate, so that enzymic hydrolysis could not account for the feeble activities of the

Table 5. *Approximate Equipotent Molar Ratios on Various Preparations of the Hybrid Series* $CH_3CO_2CH_2CH_2\overset{+}{N}Me_3$ *to* $CH_3CO_2CH_2CH_2\overset{+}{N}Et_3$

Preparation and Effect		Nature of the Cationic Group			
		$-\overset{+}{N}Me_3$	$-\overset{+}{N}Me_2Et$	$-\overset{+}{N}MeEt_2$	$\overset{+}{N}Et_3$
Cat	fall of blood pressure	1	3	400	>2000
	rise of blood pressure	1	>5	No rise	No rise
	after atropine				
Guinea pig ileum: contraction		1	2·5	700	1700
Frog heart: slowing and reduction of beat		1	2	1500	Reduces ACh effect
Rabbit auricles: slowing and reduction of beat		1	1·6	600	Reduces ACh effect
Frog rectus abdominis: contracture		1	5	300	5000

N-diethyl and N-triethyl compounds. These results suggest that the dimensions of the cationic group in acetylcholine-like compounds are critical. This conclusion is supported by the important results of WELCH and ROEFKA [54] on the phosphorus and arsenic analogues of acetylcholine (XII) and (XIII)

$CH_3CO_2CH_2CH_2\overset{+}{P}Me_3$ (XII) $CH_3CO_2CH_2CH_2\overset{+}{A}sMe_3$ (XIII)

The P–C and As–C bonds in onium salts are respectively 27·5 and 34·6 per cent longer than the N–C bond, so that the replacement of N by P or As results in a substantial increase in the volume of the onium cationic group, and in a significant decrease in typical acetylcholine-like potencies (Table 6).

Table 6.

Substance	Approximate equipotent molar ratios			
	Blood pressure (cat)	Intestinal muscle (rabbit)	Heart (frog)	Rectus abdominis (frog)
$CH_3CO_2CH_2CH_2\overset{+}{N}Me_3$	1	1	1	1
$CH_3CO_2CH_2CH_2\overset{+}{P}Me_3$	13	12	12	6
$CH_3CO_2CH_2CH_2\overset{+}{A}sMe_3$	66	90	83	37

These considerations offer some explanation of the effects of the series:

$$Me_4\overset{+}{N},\ Me_3\overset{+}{N}Et,\ Me_2\overset{+}{N}Et_2,\ Me\overset{+}{N}Et_3,\ \overset{+}{N}Et_4,$$

at the neuromuscular junction and at ganglia since the first three produce stimulation before block; the blocking effect being due to the stability of these cations and comparable with the effects of acetylcholine itself when its hydrolysis is prevented by powerful anti-acetocholinesterases. But the peculiar efficiency of benzilyloxy- and dibutylcarbamyl-ethyl dimethylethylammonium as mydriatic agents remains obscure; so also does the effectiveness of the $-NMeEt_2$ analogue of neostigmine (and of its corresponding phenol) as an anticurare agent.

At the moment no satisfactory explanation of these hybrid homologous series can be offered, although it seems likely that any explanation must involve the 'fit' of the cationic group into an anionic site at the reactive centre of the specialized region of the tissues upon which these compounds act.

One final example may be quoted, although it does not constitute a homologous series; this is the series of symmetrical cations $R_4\overset{+}{N}$, where $R = Me$, Et, *n*-Pr, or *n*-Bu. ING and WRIGHT [50] found that the order of neuromuscular blocking activity on the frog sartorius was:

$$R = Me = Bu < Pr \ll Et$$

Recently PATON and WAUD [51] have examined the depolarization effects of these four cations on the gracilis muscle of the cat. It is clear from their results that only $Me_4\overset{+}{N}$ has a depolarizing effect, so that the high blocking activity of *n*-$Bu_4\overset{+}{N}$ must be a competitive blocking effect. It seems likely that the equal blocking effect of $Et_4\overset{+}{A}s$, mentioned above, is probably due to competition with acetylcholine, although the crucial evidence is not available.

4. Conclusion

It will be clear that no general rules governing the pharmacology of homologous series, apart from the aliphatic depressants that 'obey' the Ferguson rule, can be discerned. This is not surprising when we remember the many factors that are involved in any measurement of drug action, such as the route of administration and the consequent rate of access to the ultimate site of action, the metabolic hazards which a drug must encounter, the unique properties of different tissues, etc. Moreover drugs differ quantitatively (and sometimes qualitatively) in their actions in different species and even in similar tissues of the same species. It is surprising to the chemist that pharmacologists so frequently generalize about the action of a drug from experiments on a few selected isolated tissues. It cannot be too strongly emphasized that structure-action relationships on one tissue or organ of a given species cannot be applied to

similar tissues or organs in other species. Indeed, there is much truth in CLARK's dictum ([1], p. 190) that 'every cell-drug system appears to be a law unto itself'. At the same time there is no reason to despair of finding satisfactory structure-action relationships provided that it is recognized that different cells in the same animal, and similar cells in different species, each have their own unique biochemical properties. Even with enzymes the same is true; thus BLASCHKO and HIMMS [55] examined the inhibitory action of homologous α-ω-di-*p*-amidinophenoxypolymethylenes on the amine oxidases of ten species and found a different pattern of potencies for each species. No more convincing evidence could be produced for the unique properties of living cells when a particular enzyme responds differently to members of a homologous series in different species. The pharmacologist, in so far as he is interested in discovering new medicaments for man must choose an animal that responds to drugs similarly to man; but if he is primarily interested in pharmacology as a separate scientific discipline he must try to correlate drug responses in different species with the intimate biochemistry of their species. There is no doubt that we shall never be able to understand the action of a particular drug until we know how that drug interferes with the complex biochemical reactions of the cell upon which it has its characteristic pharmacological effect. The problem is ultimately chemical: how a foreign chemical substance—the drug—interferes with the normal biochemical reactions of cells, and that living cells are so sensitive to such small physicochemical changes as successive homologues display only makes this point of view more certain.

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