grown in *Periconia*-inoculated soil or in naturally infested soil, the characteristic fungus, *P. circinata*, was readily recovered from the diseased roots.

In testing selections of milo for resistance to milo disease in the past, plant breeders were restricted in their studies to the use of soil naturally infested with the milo-disease pathogen. Such soils at times are rather heavily infested also with various other harmful fungi, whose effects on the plants under observation may be confusing. Now, however, with the use of steam-sterilized soil inoculated with the causal fungus, *Periconia circinata*, only, the resistance to milo disease will be more clearly indicated as effects of other fungi are eliminated. More complete details of these investigations will be published later.

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Nature and Spatial Relationship of the Prosthetic Chemical Groups Required for Maximal Muscarinic Action

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Dale, in his Dixon Memorial Lecture (*5*), states: "To the fundamental pharmacological problems, why a particular type of chemical structure, or more mysteriously, several apparently unrelated types, should be associated with a specific action on particular types of reactive cells, we have made no nearer approach." With this in mind we have attempted to correlate pharmacological action with certain prosthetic groups[1] which appear as a common denominator in all potent parasympathomimetic drugs. Attempts to correlate structure-activity-relationships (SAR) of chemical series of pharmacological importance have hitherto mainly considered activity as variations in chain length of aliphatic series as drawn in two dimensions. Occasionally theories have been centered around the well-known ring systems of organic chemistry. Greater correlation and understanding of SAR might be obtained by depicting formulas in three dimensions, with bond distances calculated as accurately as our present knowledge will allow. Schueler (*16*) has applied this approach successfully in relating estrogenic activity to chemical constitution. Clark (*1, 2*), from his studies and those of other workers, has amassed almost incontrovertible evidence that pharmacological action in many instances depends on the activation of receptors on the cellular surface by specific chemical prosthetic groups on the drug molecule. A cellular surface action as a possible mode of action of the muscarinic drugs is assumed in this study.

From inspection of the numerous drugs having parasympathomimetic stimulant action it becomes apparent that all contain a ketone oxygen group adjacent to an ether oxygen linkage with a methyl substituted nitrogen at a distance of two saturated carbon atoms. Assuming that these molecules have three prosthetic groups, the interprosthetic distances may be calculated for many of the known potent stimulants. The size of the acetylcholine molecule (approximately 9 A × 3 A), when compared to the size of the cell surface (20 × 1 μ for vascular smooth muscle), is infinitely small. With atomic models where 1 A equals 1 cm, the smooth muscle cell, if drawn to the same scale, would measure 2,000 × 100 m, or roughly 1 mile long and a city block in diameter. Thus, the surface of the cell containing receptors may be considered as the segment of an arc with an infinite radius, and the interprosthetic distances, for practical purposes, may be

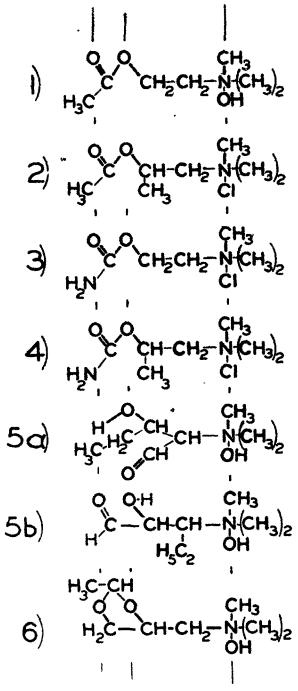

Fig. 1. Aliphatic parasympathetic stimulant drugs: (1) acetylcholine, (2) methacholine (Mecholyl), (3) carbaminoylcholine (Doryl), (4) urecholine, (5a) textbook formula for muscarine, (5b) Kögl's alternate formula for muscarine, (6) Bovet and Fourneau's acetal derivative.

regarded as linear distances and may be measured on Hirschfelder atomic models (*13*). The measured interprosthetic distances in acetylcholine are 7.0 A ketone oxygen to methyl, and 5.3 A ether oxygen to methyl (Fig. 1). Obviously, the interprosthetic distances for acetylcholine, methacholine, carbaminoylcholine, and urecholine are the same. Choline, which contains only two of the prosthetic group, has a parasympathomimetic stimulant action which is insignificant compared to that of acetylcholine. Compound 6 of Fig. 1, which has an acetal group, was

[1] The term "prosthetic group" as used in this study refers to simpler chemical complexes such as methyl groups or oxygen atoms, whereas enzymologists utilize this term for larger chemical complexes.

recently studied by Bovet and Fourneau (*6*, *7*), who found it to be potent in a dose of 0.001 mg/kg. Apparently, if the interprosthetic distances are optimal, the receptors on the cell do not differentiate between ether, ketone, ester, or acetal oxygen atoms (see Compounds 5b and 6). Hence, the nomenclature "ether oxygen" and "ketone oxygen" has been adopted in this study. Thus, it becomes plausible that parasympathomimetic stimulant action depends on adjacent prosthetic oxygen atoms at a distance of approximately 5.0 A and 7.0 A, respectively, from one or more methyl groups attached to nitrogen (Table 1). The textbook (*8*) formula for muscarine (5a

TABLE 1

SUMMARY OF MEASUREMENTS ON ATOMIC MODELS

	Distances in Angstra	
	A*	B†
Acetylcholine	5.3	7.0
(A) Muscarine	5.0	5.5
(B) Muscarine	5.0	6.7
Bovet's acetal	5.0	7.0‡
Neostigmin	7.0	5.0
(Ortho) Neostigmin	3.2	3.0
(Para) Neostigmin	7.5	8.0
Arecoline	4.0	6.0
Pilocarpine	6.0	8.0
Physostigmine	7.0	8.5
Dibutoline	5.3	7.0
Compound E-3	5.3	7.0

* Distance from center of ether oxygen to center of methyl on nitrogen.

† Distance from center of ketone oxygen to center of methyl on nitrogen.

‡ Second acetal oxygen.

of Fig. 1) is probably in error, since Kögl and his coworkers (*11*, *12*), who first elucidated the alternate formulas in 1931, have been unable over a period of 11 years to choose between the two by chemical characterization methods or synthesis. Formula 5b is more likely from this analysis of the interprosthetic distances. A physical-chemical method of measurement, such as the determination of the electron diffraction pattern of muscarine, is needed and would be most helpful.

Inspection of the aromatic group of parasympathomimetic stimulants (Fig. 2) reveals in pilocarpine, arecoline, neostigmin, and physostigmine the three fundamental prosthetic groups at the same approximate interprosthetic distances that have been postulated for acetylcholine and its aliphatic homologues. The A and B distances (Table 1) are reversed in neostigmin, which may be a further indication that the effector cell does not differentiate between ether and ketone oxygen atoms. Neostigmin was formerly thought to possess stimulant properties solely by virtue of its ability to inactivate choline esterase, but comparisons of DFP and neostigmin in animals (*15*) and in patients with myasthenia gravis (*3*) show definitely that the latter also has a direct acetylcholine-like action on striated muscles. This would be in accord with the interprosthetic distances of its three prosthetic groups.

Quaternization of the amino nitrogen of the aliphatic series invariably results in greater pharmacological potency, which may be ascribed to the greater availability of methyl on nitrogen prosthetic groups. In the aromatic series quaternization increases the pharmacological potency only when the interprosthetic distance is optimal. Thus, Stedman (*17*) has shown that the ortho and para analogues of neostigmin lose potency when quaternized, while the meta analogue (neostigmin) becomes much more potent. The benzyl urethane analogues of neostigmin (*18*) further indicate the critical nature of the optimal

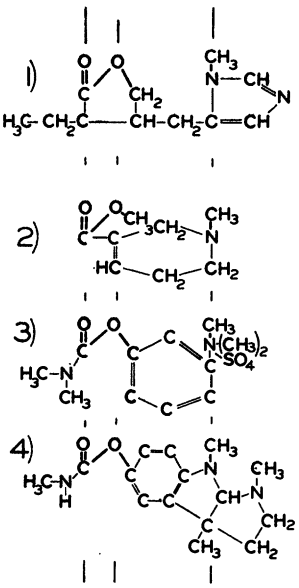

FIG. 2. Aromatic parasympathetic stimulant drugs: (1) pilocarpine, (2) arecoline, (3) neostigmin (prostigmin), (4) physostigmine.

interprosthetic distance, since in these derivatives the ortho analogue is the most potent and its activity is enhanced by quaternization while the slight activity of the meta derivative is decreased by quaternization. This may be due to the greater freedom of single methyl groups on nitrogen which makes the N to C bond distance 1.47 A available when the interprosthetic distance is too large or small. Thus, the interprosthetic distances of pilocarpine, arecoline, and physostigmine, while not optimal, may be compensated for by the fact that the methyl group is attached in each instance to a tertiary nitrogen and is freer to rotate toward or away from the oxygen prosthetic groups.

If the potent inhibitors of acetylcholine stimulation are now studied (Fig. 3), one finds again the three prosthetic groups postulated for acetylcholine action at the same or nearly the same interprosthetic distance. In these instances, however, the prosthetic groups are contained in the center ridge of a large umbrella-like molecule. This "umbrella-structure" is analogous to the blocking moieties of other blocking agents which have recently been synthesized (*i.e.* benadryl, which blocks histamine action (*19*) on various tissues, and dibenamine, which blocks most of the excitatory actions of epinephrin). The interprosthetic distances in dibutoline and

E-3 (*10*) are 7.0 A for the ketone oxygen to nitrogen methyl groups and 5.3 A for the ether oxygen to nitrogen methyl groups. Atropine interprosthetic distances cannot be calculated by the use of Hirschfelder models. A slight stimulant action should be expected from some of these agents before blocking occurs. Some evidence for this is found in the action of dibutoline on heart rate (*14*).

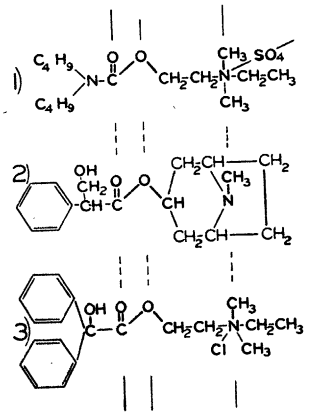

FIG. 3. Acetylcholine blocking agents: (1) dibutoline, (2) atropine, (3) Compound E-3 of Ing, Dawes, and Wajda.

Since modification or removal of the hydroxy group on the tropic acid portion of atropine analogues (*9*) decreases the degree and duration of blocking, the hydroxy group must be considered as an additional anchoring prosthetic group whose exact function is not at present clear. The simplest concept is that these blocking molecules adhere to the cell surface by means of the three or more prosthetic groups and by their continued adherence and difficult degradation prevent the smaller stimulant molecule from reaching the receptors on the cell. A second possibility which would account for the greater molar potency of atropine which blocks several mols of acetylcholine is that the umbrella-like atropine molecule may mechanically or electrostatically inactivate adjacent receptors on the cell surface so that these receptors are also unavailable for acetylcholine or other parasympathomimetic stimulants.

Other large molecules which contain only one or two of the acetylcholine prosthetic groups will show various degrees of blocking activity. Thus, methylene blue (*4*) has an atropine-like action when perfused through the isolated heart. Unlike atropine, however, methylene blue can be easily washed off the cell surfaces. The intracellular methylene blue is inactive, so that, after washing, the heart muscle, although stained blue, will again respond to acetylcholine. This phenomenon probably accounts for the mild atropine-like action of such antispasmodic drugs as Trasentin and Pavatrine, which contain only two prosthetic groups (double oxygen) and must be given in much larger doses than atropine.

Nicotinic action of the parasympathomimetic stimulants cannot be correlated at present with the prosthetic groups necessary for muscarinic action. Methyl on carbon, as in methacholine (2 of Fig. 1), apparently interferes with nicotinic action, whereas methyl on nitrogen is essential for nicotinic action (nicotine and α-lobeline).

The author realizes that distribution of active prosthetic groups, while perhaps responsible for major drug action, is strongly influenced by the associated molecular structure and the ease of degradation or detoxication of these moieties in the body. Thus, while one may be able to predict pharmacological action from prosthetic groupings, the usefulness of a new drug will depend on the pharmacological action of the associated molecular structure, which must always be determined by animal experimentation. The author further acknowledges that the present paper may contain several inaccuracies, due to the fact that interprosthetic distances have been calculated or measured from the meager data available on the interatomic bond distances of other organic compounds. The hypothesis is presented at this time with the hope that more accurate measurements may be made on these drugs by the physical-organic chemists so that pharmacologists can, in turn, define more accurately the prosthetic groups on molecules, interprosthetic distances, and the spatial relationship of the receptors on the surfaces of reacting cells. Since blocking molecules have a characteristic chemical configuration, this type of analysis may be extended to other pharmacological series (such as the barbiturates and analgesics), and the chemical nature of the hitherto unknown stimulant molecules may be closely predicted.

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