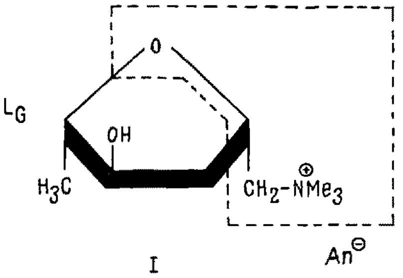
Acetylcholinesterase Inhibitory Activities of Muscarine and Muscarone Derivatives[1]

The intrinsic anticholinesterase activities of muscarine (I)[2] and of its diastereoisomers and ketonic and unsaturated derivatives[3], divorced from the complex array of physiological properties attributed through the years to crude muscarinic extracts, were determined for the first time in this investigation by a constant-pH titration technique[4] with the acetylcholine chloride-acetylcholinesterase system (AC-AChE) operating at optimum $(3{\cdot}33 \times 10^{-3}\,M)$ substrate level, pH 7·4 and 25·14°C, and an enzyme preparation from electric eel tissue assaying at $4{\cdot}18 \times 10^3$ μmoles AC hydrolyzed/h/mg dry protein.



I

Sufficient compound was available for only one kinetic inhibition experiment with the majority of samples on hand, but in two instances, where inhibition runs at two levels of concentration were permitted, an average reproducibility of inhibitory strength indices within a 20% spread was observed. The results, expressed as competitive enzyme-inhibitor dissociation constants K_I (with decreasing values implying greater strength), are summarized in the Table.

Several interesting points emerge from these data. First, the mean inhibitory activity of the entire sequence is just about equivalent[5] to that of choline $(K_I \simeq 4{\cdot}5 \times 10^{-4})$, which might perhaps be expected from the close generic relationship between choline and the dotted portion of the structure I. However, more striking is the observation that the muscarine isomers group into two classes of inhibitory potency. The parent series exemplified by I and II is of the order of *three* times as powerful in AChE-AC inhibition as the isomers in the *allo* series (III and IV). In the 4, 5-dehydromuscarines V and VI potency is at the same level as the muscarines themselves, and the enzyme clearly distinguishes between *cis* and *trans* configurations.

Oxidation of the secondary alcohol function in I to muscarone (VII) is of little or no effect on inhibitory strength, while the corresponding transition in the *allo* series III→VIII leads to quite a pronounced *increase* in power. In sharp contrast, the same transition in the dehydromuscarone series VI→IX leads to an abrupt *decrease* in activity. Viewed as a group, the normal and *allo* muscarones (VII and VIII) are nearly equal in power, and do not evoke the same degree of stereochemical differentiation on the part of the enzyme, as the normal and *allo* isomers in the muscarine series (I and II vs. III and IV).

The most potent inhibitory compound in the entire sequence is the O-acetylmuscarine X. Yet, this ester is essentially untouched as a substrate by the enzyme, in line with the well known observation of decrease in cata-

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[1] The opinions in this paper are those of the authors and do not necessarily reflect the views of the Navy Department or the naval service at large.

[2] The correct way to represent the absolute configuration of L-muscarine [E. HARDEGGER and F. LOHSE, Helv. chim. Acta *40*, 2383 (1957] is shown in I which pictures it as N-[1,3,6-trideoxy-2,5-anhydro-L-*ribo*-hexityl]-N-trimethylammonium halide. The Rosanov carbon is C(5) and has the LG-configuration.

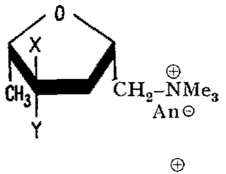
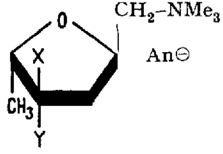
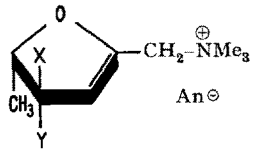
[3] C. H. EUGSTER, F. HÄFLIGER, R. DENSS, and E. GIROD, Helv. chim. Acta *41*, 205, 583, 705 (1958); Helv. chim. Acta *42* (1959), in press. – We are greatly indebted to Dr. F. HÄFLIGER, J. R. Geigy, Basle, for placing these compounds at our disposal.

[4] S. L. FRIESS, A. A. PATCHETT, and B. WITKOP, J. Amer. chem. Soc. *79*, 459 (1957).

[5] I. B. WILSON, J. biol. Chem. *197*, 215 (1952).

Table

Inhibition of AChE-AC by Muscarine Congeners

Structural Formula				Compounds	Inhibitory Strength expressed as Competitive $K_I \cdot 10^4$
	*I*	$x = \mathrm{OH}$	$y = \mathrm{H}$	DL muscarine chloride	2,2
	II	$x = \mathrm{H}$	$y = \mathrm{OH}$	DL epi-muscarine chloride or iodide	3,0[a]
	VII	$x =$	$y = \mathrm{O}$	DL muscarone iodide	2,5
	X	$x = \mathrm{OAc}$	$y = \mathrm{H}$	DL acetylmuscarine iodide[b]	0,76
	III	$x = \mathrm{OH}$	$y = \mathrm{H}$	DL allo-muscarine chloride or iodide	7,8[a]
	IV	$x = \mathrm{H}$	$y = \mathrm{OH}$	DL epiallo-muscarine chloride	10,1
	VIII	$x =$	$y = \mathrm{O}$	DL allo-muscarone iodide	1,6
	V	$x = \mathrm{H}$	$y = \mathrm{OH}$	DL 4,5-dehydromuscarine iodide (*cis*)	3,4
	VI	$x = \mathrm{OH}$	$y = \mathrm{H}$	DL 4,5-dehydromuscarine iodide (*trans*)	1,9
	IX	$x =$	$y = \mathrm{O}$	DL 4,5-dehydromuscarone iodide	10,9

[a] Multiple value average with a reproducibility within 20%.

[b] Proved to be completely inactive as a substrate, before testing as an inhibitor of AC hydrolysis.

lytic activity with increasing distance between –OAc and –NMe$_3$ functions beyond that of a single –CH$_2$CH$_2$– unit.

$^+$Recent pharmacological data for these muscarine derivatives[6,7] show that the muscarines are quite stereospecific in their actions on a diversity of pharmacological preparations, whereas the muscarones are not. This parallels the present findings that the enzyme responds *in vitro* more sensitively to stereochemical differences in the muscarine than in the muscarone series. In contrast to this parallel, the muscarones are much more active in stimulating smooth muscle[7] and with respect to intrinsic nicotinic power[6,7] than are the muscarines.

The examination of Stuart-Briegleb models of the muscarine isomers leads to one interesting possibility for differentiation. In the natural series as compared with *allo*, there appears to be a closer approach to coplanarity of the hetero ring with both the quaternary N and hydroxyl O atoms, which could in turn lead to additional binding strength in the muscarines if the ring (through its high electron-density locus) supplements the normal bifunctional[8] binding through the two polar groups. This would imply that the subtle changes in AChE binding strengths observed for these compounds are the result of the net steric influences on 3-pointed binding afforded by the three substituted ring positions, and directly mirrors the corresponding conclusions of WASER[6] regarding the geometry of interactions with the cholinergic receptor.

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National Institute of Arthritis and Metabolic Diseases, National Institutes of Health and Naval Medical Research Institute**, Bethesda (Md.), April 28, 1959.*

Zusammenfassung

Die Anticholinesterase-Wirkung von synthetischem D,L-Muscarin und weiteren 9 Derivaten wurde an einem hochgereinigten, aus *Electrophorus electricus* gewonnenen Enzympräparat durch Titration bei konstantem pH ermittelt. Als wirksamster Hemmstoff erwies sich O-Acetyl-D,L-muscarin, das als Substrat ungeeignet ist. Die enzymatischen werden mit den pharmakologischen Befunden verglichen.

[6] P. G. WASER, Exper. *14*, 356 (1958).

[7] L. GYERMEK and K. R. UNNA, paper presented to the American Society for Pharmacology and Experimental Therapeutics, Aug. 25, 1958, Ann Arbor (Mich.).

[8] D. NACHMANSOHN and I. B. WILSON, Adv. Enzymol. *12*, 259 (1951).

Preliminary Note on Certain Pharmacological Properties of 3β,16α-Dihydroxyallopregnan-20-one

Recently 3β,16α-dihydroxyallopregnan-20-one has been isolated from hog adrenals (NEHER, DESAULLES, VISCHER, WIELAND, and WETTSTEIN[1]) as well as from the urine in certain human cases of adreno-genital syndrome of the salt-losing type (NEHER, MEYSTRE, WETTSTEIN[2]). As it could be supposed that this compound might be responsible for the augmented salt loss observed in this type of syndrome (WILKINS and LEWIS[3], KLEIN *et al.*[4]), we first studied its effects in water and salt metabolism. The present note is intended to give an account on this particular aspect of its action.

Methods. All experiments were performed on adrenalectomised rats of the same breed, in order to avoid strain-bound differences in sensitivity. The method used has been described in detail elsewhere (DESAULLES and MEIER[5]), the only difference being that instead of collecting urine from the 4–6th h after treatment and salt loading (I), we collected it immediately after loading and treatment for 4 h consecutively (II) (Fig. 1).

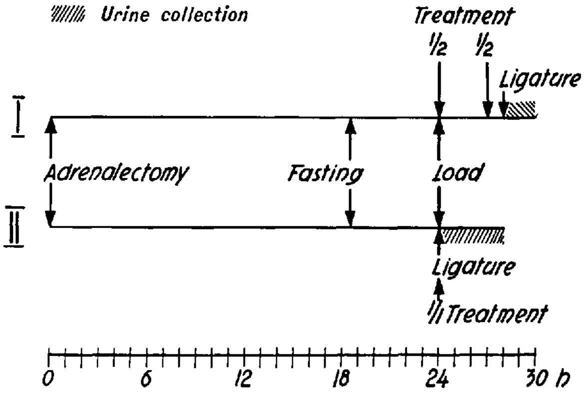

Fig. 1.—Diagrams of the experimental procedure.

The substance to be tested was given by intramuscular injection in a dose-range varying from 0·2 to 25 µg/kg.

Experimental results involve a collective of over 550 rats. We have compared the sodium excretion of treated animals with that of untreated control animals studied simultaneously (Fig. 2).

Sodium excretion in control animals varies over a wide range and shows a typical one-tailed form of distribution characteristic for our animal colony. Nevertheless, most of the individual values obtained are smaller than 6·0 mg%/4^h. The mean value amounts to 4·82 mg%/4^h.

Treatment with 3β,16α-dihydroxyallopregnan-20-one enhances sodium excretion. The mean values of the treated groups rise moderately at the 0·2 µg/kg dose level (mean amounting to 7·89 mg%/4^h) and attain maximal intensity at a dosage of 1·0 µg/kg (mean amounting to 9·38 mg%/4^h). Higher dose levels of the substance cause no further increase in sodium excretion, the mean for which remains practically at the same level as that produced by the injection of 1·0 µg/kg of the substance.

When the individual values are examined, it will be seen that they are more evenly distributed: the total number of high sodium values increases consistently, while the number of low excretion values diminishes but does not disappear completely, about one-third of the animals showing no effect on sodium excretion following treatment with even the highest dosage. 3β,16α-dihydroxyallopregnan-20-one does not significantly affect

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[1] R. NEHER, P. DESAULLES, E. VISCHER, P. WIELAND, and A. WETTSTEIN, Helv. chim. Acta *61*, 1667 (1958).

[2] R. NEHER, CH. MEYSTRE, and A. WETTSTEIN, Helv. chim. Acta *62*, 132 (1959).

[3] L. WILKINS and R. A. LEWIS, Trans. 17th Meet. Conf. on Metab. Aspects of Convalescence (J. Macy Jr. Found., New York 1948), p. 168.

[4] R. KLEIN, P. TAYLOR, C. PAPADATOS, Z. LARON, D. KEELE, J. FORTUNATO, C. BYERS, and C. BILLINGS, Proc. Soc. exp. Biol. Med. *98*, 863 (1958).

[5] P. DESAULLES and R. MEIER, Schwz. Med. Wschr. *86*, 1060 1956).