

Summary

In order to determine the site of action of organophosphate group on acetylcholine (ACh) receptor surface, the effect of several antagonists of ACh has been observed with Magnus method using the isolated intestine of mice. These experiments gave following results :

1) A modified method for determining the site of action of antagonist on ACh receptor surface was established stochastically and the validity was evidenced by two trial experiments.

2) According to this justified method, the anticholinergic effect of four pairs consisting of two antagonists were demonstrated and from these results, four pairs were divided into groups acting on the same site and on the different site; the former is two pairs consisting of pyridine aldoxime methiodide (PAM) and atropine, and parathion and isopentyl acetate, the latter is two pairs consisting of atropine and parathion, and parathion and PAM. From this fact, it was concluded that organophosphate group of parathion is attracted to, what is called, the esteratic site of ACh receptor surface in the inhibitory action against ACh.

3) From the fact that the interaction between PAM and butyl diethyl phosphate is recognized but PAM and isopropyl phosphate are independent each other in the inhibitory action against ACh, it was suggested as the possible mechanism that the butyl radical of phosphate can be to hinder the approach of PAM to the anionic site of ACh receptor surface. This is an experimental evidence that there are two active sites with the distance of 7 Å on ACh receptor surface.

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**22. Masayasu Kimura^{*1} and Isamu Saikawa^{*2} : Molecular Pharmacological Studies on Drug-Receptor Complexes System in Drug Action. IV.^{*3}
Relationship between Anticholinergic and Anticholinesterase
Activities of Organophosphoryl Choline Derivatives
based on their Chemical Structure.^{*4}**

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In an early series of study on organophosphorus compounds, Kimura,¹⁾ one of the authors, found that parathion inhibits acetylcholine (ACh) action on smooth muscle in addition to anticholinesterase (ChE) action, and then with his co-workers he²⁾ confirmed that ACh molecule is competitively inhibited by parathion molecule at ACh receptor. It has been experimentally concluded by means of pharmacological method that substance

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^{*3} Part III : This Bulletin, 12, 150 (1964).

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1) M. Kimura : This Bulletin, 11, 44 (1963).

2) M. Kimura, T. Igarashi, S. Iwashita : *Ibid.*, 11, 51 (1963).

containing organophosphate group occupies the esteratic site of the receptor surface in order to inhibit ACh action on peripheral nervous system.³⁾

On the other hand, it has been well known since Clark's discovery⁴⁾ that quaternary ammonium salt plays an important role in cholinergic action and that anticholinergic drugs containing it occupies the anionic site of ACh receptor surface. Therefore, compounds which have both a quaternary ammonium group and an organophosphate group at a distance of about 7 Å in its molecule, *e.g.* organophosphoryl choline, will be expected to display the anticholinergic action. From a pharmacological observation, insofar as we know, almost all reports were concerned with nothing else but with inhibitors of ChE and their toxicity^{5~9)} or, at the most, only the relationship of pharmacological action to anti-ChE activity.^{10~14)}

In the present paper, the synthesis of known and unknown compounds of organophosphoryl choline derivatives was reported and the relationship between anticholinergic and anti-ChE activities were studied. The purpose of this study was to provide against a clue to some differences between the active site on the surface of ACh receptor and ChE, and then, if possible, finding more potent anticholinergic compounds, further evidence can be furnished as a proof of an estimation method for determining the site of action of drug in the previous paper.³⁾

Method and Materials

1) **Estimation for Potency of Biological Activity**—For determination of curare-like action, Magnus method was adapted. The rectus abdominis of frog, weighing about 30 g., was used to test the compounds.

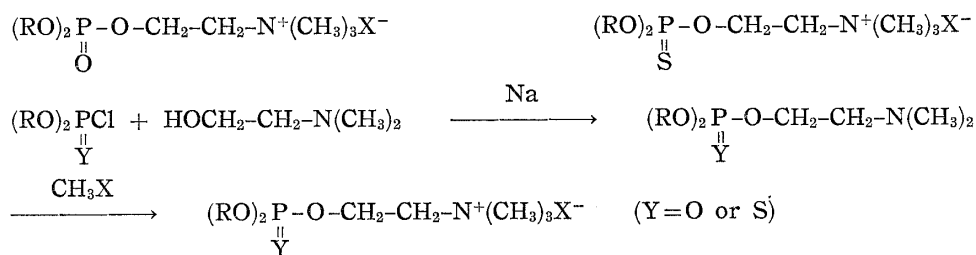
For assay of atropine-like action, Magnus method was also adapted¹⁵⁾ using intestinal segments, 20~25 mm. long, isolated from mice, weighing 15~20 g.

For *in vitro* measurement of anti-ChE activity, Warburg manometric method was used for the inhibition of ChE with enzyme preparation from rabbit's serum.¹⁾

These potencies were expressed as mole concentration at 50% inhibitory effect against a given doses of ACh (in above cases 1.1×10^{-5} , 5.5×10^{-7} , and $1.1 \times 10^{-3}M$ respectively) and calculated with Litchfield and Wilcoxon's method.¹⁶⁾

2) **Compounds of Organophosphoryl Choline Derivatives**—The synthesis of these compounds were conducted as follows;

a) Phosphate and thiono-phosphate type :



- 3) M. Kimura : This Bulletin, **12**, 150 (1964).
- 4) A. J. Clark : J. Physiol., **64**, 123 (1927).
- 5) L. E. Tammelin : Acta Chem. Scand., **11**, 856 (1957).
- 6) U. S. Patent; 2911430.
- 7) L. E. Tammelin : Arkiv Kemi, **12**, 287 (1958).
- 8) A. W. D. Avison : Chem. & Ind. (London), **1954**, 288.
- 9) T. Fredriksson : Arch. intern. Pharmacodynamie, **113**, 101 (1959).
- 10) G. B. Koelle, E. C. Steiner, H. H. Wagener, S. Smart : J. Pharmacol. Exptl. Therap., **118**, 420 (1956).
- 11) T. Fredriksson : Arch. intern. Pharmacodynamie, **115**, 474 (1958).
- 12) W. Schaumann, G. Lob : J. Pharmacol. Exptl. Therap., **123**, 114 (1958).
- 13) R. Hazard, J. Cheymol, P. Chabrier, A. Carayon-Gentil : Comt. rend., **243**, 2180 (1956).
- 14) R. D. O'Brien : J. Econ. Entomol., **52**, 812 (1959).
- 15) K. Takagi, M. Kimura : This Bulletin, **4**, 444 (1956).
- 16) J. T. Litchfield, F. Wilcoxon : J. Pharmacol. Exptl. Therap., **96**, 99 (1949).

b) Thiol-phosphate and thionoithiol-phosphate type :

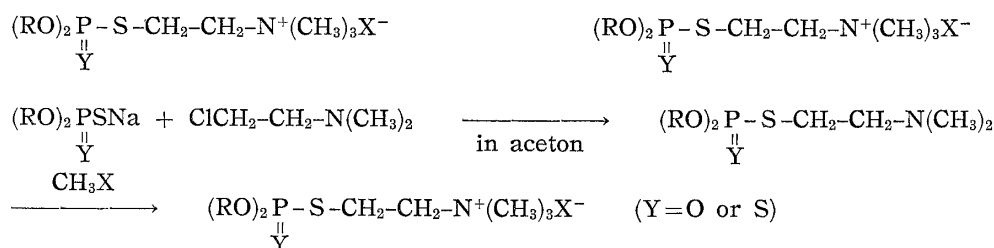
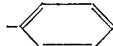
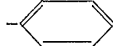
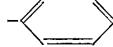
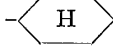
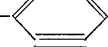
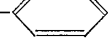
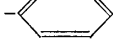


Table I lists nineteen synthetic compounds, their structure and properties.

TABLE I. Structure and Properties of Organophosphoryl Choline Derivatives

$ \begin{array}{c} \text{R}_1\text{O} \\ \diagup \\ \text{P}-\text{Z}-\text{CH}_2-\text{CH}_2-\text{N}^+(\text{CH}_3)_3\text{X}^- \\ \diagdown \\ \text{R}_2\text{O} \\ \text{Y} \end{array} $							
Signal No.	R ₁	R ₂	Y	Z	X	m.p. (°C)	Reference
NP-211 (I)	CH ₃	CH ₃	O	O	I	130~135	8
NP-212 (I)	C ₂ H ₅	C ₂ H ₅	O	O	I	95~97.5	8
NP-221 (I)	CH ₃	CH ₃	S	O	I	92	8
NP-222 (I)	C ₂ H ₅	C ₂ H ₅	S	O	I	113~116	8
NP-222 (Br)	C ₂ H ₅	C ₂ H ₅	S	O	Br	90.5~94	8
NP-224 (I)			S	O	I	95~99	C ₁₇ H ₂₃ O ₃ NSPI { N: 2.92 3.17 P: 6.47 6.23
NP-224 (Br)			S	O	Br	113~118	
NP-222, 4 (I)	C ₂ H ₅		S	O	I	97~100	C ₁₃ H ₂₃ O ₃ NSPI { N: 3.25 3.12 P: 7.19 6.80
NP-222, 4 (Br)	C ₂ H ₅	CH(CH ₃) ₂	S	O	Br	101.5~106	
NP-232 (I)	C ₂ H ₅	C ₂ H ₅	O	S	I	120	8
NP-233 (I)	CH(CH ₃) ₂	CH(CH ₃) ₂	O	S	I	141~143.5	8
NP-242 (I)	C ₂ H ₅	C ₂ H ₅	S	S	I	101~102	8
NP-242 (Br)	C ₂ H ₅	C ₂ H ₅	S	S	Br	96~99	8
NP-243 (I)	CH(CH ₃) ₂	CH(CH ₃) ₂	S	S	I	124~125	8
NP-243 (Br)	CH(CH ₃) ₂	CH(CH ₃) ₂	S	S	Br	132	8
NP-244 (I)			S	S	I	103~106	C ₁₇ H ₂₃ O ₂ NS ₂ PI { N: 2.83 3.09 P: 6.26 6.85
NP-245 (I)			S	S	I	172	C ₁₇ H ₂₇ O ₂ NS ₂ PI { N: 2.76 2.84 P: 6.11 5.98
NP-244pC (I)			S	S	I	159~160	C ₁₇ H ₂₁ O ₂ NS ₂ PCl ₂ I { N: 2.48 2.58 P: 5.50 4.85
NP-244pB (I)			S	S	I	183~186	C ₁₇ H ₂₁ O ₂ NS ₂ PBr ₂ I { N: 2.14 2.16 P: 4.75 4.49

Results

I. Comparison of Potency in Curare-like Action of Phosphorylcholine Derivatives

In Table II, the mole concentration of ID₅₀*⁵ were estimated by the experimental design of 2 doses and 4 repeats.

II. Comparison of Potency of Atropine-like Action of Organophosphoryl Choline Derivatives

In Table III, the mole concentration of ID₅₀ was estimated by the experimental design of 2 doses and 4 repeats.

*⁵ ID₅₀ : 50% inhibitory dose

TABLE II. ID₅₀ of Organophosphoryl Choline Derivatives in Curare-like Action

Signal No.	ID ₅₀ (M)	Confidence limits	Potency ratio % (for 100 of <i>d</i> -tubocurarine)
NP-211 (I)	no action by itself, enhanced ACh action		
NP-212 (I)	no action at $8.85 \times 10^{-4}M$, ACh-like action at $2.65 \times 10^{-3}M$		
NP-221 (I)	ACh-like action at $5.42 \times 10^{-4}M$		
NP-222 (I)	1.52×10^{-4}	$5.82 \times 10^{-5} \sim 3.92 \times 10^{-4}$	0.68
NP-222 (Br)	9.4×10^{-5}	$2.76 \times 10^{-5} \sim 3.20 \times 10^{-4}$	1.10
NP-224 (I)	1.07×10^{-6}	$4.89 \times 10^{-7} \sim 2.34 \times 10^{-6}$	92.2
NP-224 (Br)	1.06×10^{-6}	$4.06 \times 10^{-7} \sim 2.77 \times 10^{-6}$	97.1
NP-222, 4 (I)	9.00×10^{-6}	$4.05 \times 10^{-6} \sim 2.00 \times 10^{-5}$	11.4
NP-222, 4 (Br)	1.00×10^{-5}	$4.22 \times 10^{-6} \sim 2.30 \times 10^{-5}$	10.3
NP-232 (I)	no action by itself, enhanced ACh at $10^{-7} \sim 10^{-4}M$		
NP-233 (I)	6.8×10^{-5}	$2.75 \times 10^{-5} \sim 1.69 \times 10^{-4}$	1.52
NP-242 (I)	2.5×10^{-5}	$7.72 \times 10^{-6} \sim 8.10 \times 10^{-5}$	4.12
NP-242 (Br)	2.77×10^{-5}	$9.86 \times 10^{-6} \sim 7.78 \times 10^{-5}$	3.72
NP-243 (I)	7.20×10^{-6}	$2.72 \times 10^{-6} \sim 1.91 \times 10^{-5}$	14.3
NP-243 (Br)	5.2×10^{-6}	$1.60 \times 10^{-6} \sim 1.69 \times 10^{-5}$	19.8
NP-244 (I)	6.9×10^{-7}	$2.38 \times 10^{-7} \sim 2.00 \times 10^{-6}$	149
NP-245 (I)	4.2×10^{-7}	$1.29 \times 10^{-8} \sim 1.37 \times 10^{-7}$	243
NP-244pC (I)	1.13×10^{-7}	$4.18 \times 10^{-8} \sim 3.05 \times 10^{-7}$	911
NP-244pB (I)	4.22×10^{-7}	$5.70 \times 10^{-8} \sim 3.12 \times 10^{-6}$	244
<i>d</i> -Tubocurarine	1.03×10^{-6}	$3.12 \times 10^{-7} \sim 3.40 \times 10^{-6}$	100

TABLE III. ID₅₀ of Organophosphoryl Choline Derivatives in Atropine-like Action

Signal No.	ID ₅₀ (M)	Confidence limits	Potency ratio % (for 100 of atropine)
NP-211 (I)	more than $5 \times 10^{-5}M$		
NP-212 (I)	more than $5 \times 10^{-5}M$		
NP-221 (I)	more than $5 \times 10^{-5}M$		
NP-222 (I)	1.90×10^{-5}	$8.68 \times 10^{-6} \sim 4.16 \times 10^{-5}$	0.03
NP-224 (I)	6.43×10^{-6}	$2.47 \times 10^{-6} \sim 1.67 \times 10^{-5}$	0.10
NP-222, 4 (I)	1.16×10^{-5}	$3.93 \times 10^{-6} \sim 3.42 \times 10^{-5}$	0.05
NP-232 (I)	ACh-like action at $2.7 \times 10^{-5}M$		
NP-233 (I)	more than $5 \times 10^{-5}M$		
NP-242 (I)	2.26×10^{-5}	$8.53 \times 10^{-6} \sim 5.99 \times 10^{-5}$	0.03
NP-243 (I)	7.70×10^{-6}	$3.52 \times 10^{-6} \sim 1.69 \times 10^{-5}$	0.07
NP-244 (I)	4.91×10^{-7}	$2.05 \times 10^{-7} \sim 1.18 \times 10^{-6}$	1.28
NP-245 (I)	6.09×10^{-6}	$2.77 \times 10^{-6} \sim 1.34 \times 10^{-5}$	0.10
NP-244pC (I)	4.29×10^{-6}	$2.32 \times 10^{-6} \sim 7.71 \times 10^{-6}$	0.15
NP-244pB (I)	5.25×10^{-6}	$3.04 \times 10^{-6} \sim 9.08 \times 10^{-6}$	0.12
Atropine	6.29×10^{-9}	$2.47 \times 10^{-9} \sim 1.60 \times 10^{-8}$	100

III. Comparison of Potency of Anti-Cholinesterase Actions of Various Organophosphoryl Choline Derivatives

In Table IV, the mole concentrations of ID₅₀ were estimated by the experimental design of 2 doses and 4 repeats.

IV. Influence of Neostigmine upon Curare-like Action of Organophosphoryl Choline Compounds

In view of the antagonistic action of neostigmine on *d*-tubocurarine, its influence on organophosphoryl choline was observed. Table V shows the comparative potency of curare-like action of organophosphoryl choline in the presence of neostigmine, $3 \times 10^{-5}M$. These potencies were estimated by an experimental design of 2 doses and 4 repeats.

TABLE IV. ID₅₀ of Organophosphoryl Choline Derivatives in Anticholinesterase Action

Signal No.	ID ₅₀ (M)	Confidence limits	Potency ratio % (for 100 of neostigmine)
NP-211 (I)	9.73×10^{-3}	$2.32 \times 10^{-3} \sim 4.09 \times 10^{-2}$	almost 0
NP-212 (I)	1.25×10^{-3}	$2.02 \times 10^{-4} \sim 7.75 \times 10^{-3}$	0.01
NP-221 (I)	3.80×10^{-4}	$1.19 \times 10^{-4} \sim 1.22 \times 10^{-3}$	0.05
NP-222 (I)	3.05×10^{-5}	$4.49 \times 10^{-6} \sim 2.07 \times 10^{-4}$	0.57
NP-224 (I)	5.11×10^{-6}	$1.25 \times 10^{-6} \sim 2.10 \times 10^{-5}$	3.39
NP-222, 4 (I)	5.57×10^{-5}	$6.55 \times 10^{-6} \sim 4.73 \times 10^{-5}$	0.31
NP-232 (I)	1.98×10^{-6}	$4.04 \times 10^{-7} \sim 9.70 \times 10^{-6}$	8.77
NP-233 (I)	1.86×10^{-6}	$4.05 \times 10^{-7} \sim 1.06 \times 10^{-5}$	9.26
NP-242 (I)	6.77×10^{-5}	$1.54 \times 10^{-5} \sim 2.98 \times 10^{-4}$	0.26
NP-243 (I)	1.78×10^{-3}	$3.63 \times 10^{-4} \sim 8.72 \times 10^{-3}$	0.01
NP-244 (I)	9.49×10^{-5}	$1.51 \times 10^{-5} \sim 5.98 \times 10^{-4}$	0.18
NP-245 (I)	5.52×10^{-5}	$8.49 \times 10^{-6} \sim 3.59 \times 10^{-4}$	0.31
NP-244pC (I)	2.38×10^{-5}	$4.87 \times 10^{-6} \sim 1.17 \times 10^{-4}$	0.73
NP-244pB (I)	9.19×10^{-6}	$9.28 \times 10^{-7} \sim 9.10 \times 10^{-5}$	1.88
Neostigmine	1.73×10^{-7}		100

TABLE V. ID₅₀ of Curare-like Action of Organophosphoryl Choline in the Presence of Neostigmine

	ID ₅₀ (M)	Confidence limits	Potency ratio
<i>d</i> -Tubocurarine	9.93×10^{-6}	$1.66 \times 10^{-6} \sim 5.96 \times 10^{-5}$	1
NP-244 (I)	1.39×10^{-6}	$5.32 \times 10^{-7} \sim 3.63 \times 10^{-6}$	7.14
NP-245 (I)	4.83×10^{-7}	$9.66 \times 10^{-8} \sim 2.42 \times 10^{-6}$	20.6

Discussion and Conclusions

From the comparison of potency in curare-like action of organophosphoryl choline derivatives, it was shown that the compounds of phosphate and thiol-phosphate type showed little activity as a curare-like action, but enhanced acetylcholine (ACh) action or exhibited some of ACh-like action. Except for NP-221(I), almost all of the thionophosphates and thionothiol-phosphates exhibited more or less a curare-like action. From the data given in Table II, it is readily seen that in the structure (RO)₂P-Z-CH₂-CH₂-

N⁺(CH₃)₃X⁻, the R radical increases the potency of the curare-like action according to the order of CH₃<C₂H₅<CH(CH₃)₂< <  < -X and even in the com-

bination of two different Rs, their order remains that of -P(OC₂H₅)₂, -P(OC₂H₅)()₂, and -P()(),

and -P()(). This means that the size of the R radical plays an important

role in curare-like action of organophosphoryl choline. As for quaternary ammonium salts, their bromides were generally more potent than iodide salts but the difference was very slight. In the curare-like action of this series of compounds, it was found that NP-244(I), NP-245(I), NP-244pC(I) and NP-244pB(I) were more potent than *d*-tubocurarine. These four compounds may be introduced as curare-like substances of a new type.

From the comparison of potency in atropine-like action of organophosphoryl choline, it was shown that almost all compounds have more or less atropine-like action except

for NP-232(I) which has a lower potency value than that of atropine, and at the most, only NP-244(I) had about one per cent of the potency of atropine. Therefore, it is concluded that these compounds have little atropine-like action. However, the relations between its structure and potency indicate a tendency for curare-like action.

From the comparison of potency in anticholinesterase (anti-ChE) action of organophosphoryl choline, it was shown that all compounds have more or less anti-ChE action without exception, but their potency was much lower than that for neostigmine. From the data given Table IV, it was shown that in the relations of structure to potency, thiol-phosphate type was the most potent and thiono-phosphate type was more potent than the thionothiol-phosphate type. This comparison is interesting to note that the anti-ChE action was the reverse order of curare-like action. R group dose not always enhance potency by the same manner as the curare-like action.

Such as the example of this results, it is interesting to note that when some compound had two or more different types of action these actions were distinguished by its certain concentration. It is possible to discuss this matter in technical terms "selectivity of these action" with respect to these compounds. For this purpose, the selective indexes of organophosphoryl choline derivatives between curare-like and atropine-like actions and those between curare-like and anti-ChE actions were calculated from the data presented in Tables II, III, and IV, and the results were shown in Table V. This selective index is represented by the negative logarithmic coefficient of the ratio of ID_{50} of the two actions.

TABLE V. Selective Indexes of Organophosphoryl Choline represented
by $-\log \frac{ID_{50} \text{ of one action}}{ID_{50} \text{ of another action}}$

Signal No.	<u>Atropine action</u> Curare action	<u>Anti-ChE action</u> Curare action
NP-222(I)	-0.90	-0.70
NP-224(I)	0.78	0.68
NP-222, 4(I)	1.01	1.70
NP-233(I)	-0.13	-1.56
NP-242(I)	-0.05	0.43
NP-243(I)	0.03	2.38
NP-244(I)	0.15	2.14
NP-245(I)	1.16	2.12
NP-244pC(I)	1.58	2.33
NP-244pB(I)	1.10	1.34

From Table V it was shown that the absolute coefficients of the selective indexes between curare-like and atropine-like action were relatively low compared to those between curare-like and anti-ChE actions. This difference was illustrated by the degree of resemblance between the sites of action. Since there was almost a parallel relation between the curare-like and atropine-like actions in regards to structure and activity, the selective index of this pair increased gradually according to the size of the R radical, suggesting that this was due to the physicochemical factor of R radical in penetrating into different biophases surrounding the ACh receptor in skeletal and smooth muscles. On the contrary, in the selective indexes between curare-like and anti-ChE actions only the thionothiol-phosphate type was markedly greater than the other type. Because, this is due to the difference between strong curare-like and weak ChE actions. This fact can clearly point out the difference between the nature of surfaces of ACh receptor and ChE. On the other hand, from the results shown in Table V, the action

