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 he[2] 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

*1 5-Okuda, Toyama (木村正康).
*2 8-Shimookui, Toyama (才川 勇).
*3 Part III : This Bulletin, 12, 150 (1964).
*4 This study was supported in part by Grant-in-Aid for Fundamental Scientific Research from the Ministry of Education, and presented at the 81th annual meeting of the Pharmaceutical Society of Japan (July 1961, in Sapporo).
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 discovery[4] 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.

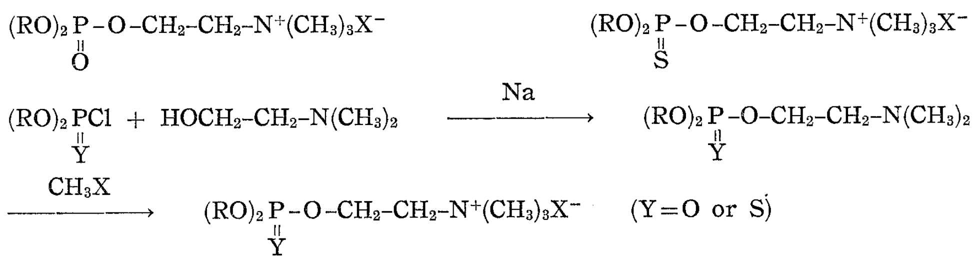
For assay of atropine-like action, Magnus method was also adapted[15] using intestinal segments, 20~25 mm. long, isolated from mice, weighing 15~20 g.

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 $1.1\times10^{-3}M$ 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. Lichifield, F. Wilcoxon : J. Pharmacol. Exptl. Therap., **96**, 99 (1949).

b) Thiol-phosphate and thionothiol-phosphate type :

$$(RO)_2\underset{\underset{Y}{\|}}{P}-S-CH_2-CH_2-N^+(CH_3)_3X^- \qquad (RO)_2\underset{\underset{Y}{\|}}{P}-S-CH_2-CH_2-N^+(CH_3)_3X^-$$

$$(RO)_2\underset{\underset{Y}{\|}}{P}SNa + ClCH_2-CH_2-N(CH_3)_2 \xrightarrow[\text{in aceton}]{} (RO)_2\underset{\underset{Y}{\|}}{P}-S-CH_2-CH_2-N(CH_3)_2$$

$$\xrightarrow{CH_3X} (RO)_2\underset{\underset{Y}{\|}}{P}-S-CH_2-CH_2-N^+(CH_3)_3X^- \qquad (Y=O \text{ or } S)$$

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

TABLE I. Structure and Properties of Organophosphoryl Choline Derivatives

$$\begin{matrix} R_1O \\ R_2O \end{matrix}\!\!>\underset{\underset{Y}{\|}}{P}-Z-CH_2-CH_2-N^+(CH_3)_3X^-$$

Signal No.	R_1	R_2	Y	Z	X	m.p. (°C)	Reference		Calcd.	Found
NP-211 (I)	CH_3	CH_3	O	O	I	130~135	8			
NP-212 (I)	C_2H_5	C_2H_5	O	O	I	95~97.5	8			
NP-221 (I)	CH_3	CH_3	S	O	I	92	8			
NP-222 (I)	C_2H_5	C_2H_5	S	O	I	113~116	8			
NP-222 (Br)	C_2H_5	C_2H_5	S	O	Br	90.5~94	8			
NP-224 (I)	$-C_6H_5$	$-C_6H_5$	S	O	I	95~99	$C_{17}H_{23}O_3NSPI$	N: P:	2.92 6.47	3.17 6.23
NP-224 (Br)	$-C_6H_5$	$-C_6H_5$	S	O	Br	113~118				
NP-222, 4 (I)	C_2H_5	$-C_6H_5$	S	O	I	97~100	$C_{13}H_{23}O_3NSPI$	N: P:	3.25 7.19	3.12 6.80
NP-222, 4 (Br)	C_2H_5	$CH(CH_3)_2$	S	O	Br	101.5~106				
NP-232 (I)	C_2H_5	C_2H_5	O	S	I	120	8			
NP-233 (I)	$CH(CH_3)_2$	$CH(CH_3)_2$	O	S	1	141~143.5	8			
NP-242 (I)	C_2H_5	C_2H_5	S	S	I	101~102	8			
NP-242 (Br)	C_2H_5	C_2H_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)	$-C_6H_5$	$-C_6H_5$	S	S	I	103~106	$C_{17}H_{23}O_2NS_2PI$	N: P:	2.83 6.26	3.09 6.85
NP-245 (I)	$-C_6H_{11}$ (H)	$-C_6H_{11}$ (H)	S	S	I	172	$C_{17}H_{27}O_2NS_2PI$	N: P:	2.76 6.11	2.84 5.98
NP-244pC (I)	$-C_6H_4-Cl$	$-C_6H_4-Cl$	S	S	I	159~160	$C_{17}H_{21}O_2NS_2PCl_2I$	N: P:	2.48 5.50	2.58 4.85
NP-244pB (I)	$-C_6H_4-Br$	$-C_6H_4-Br$	S	S	I	183~186	$C_{17}H_{21}O_2NS_2PBr_2I$	N: P:	2.14 4.75	2.16 4.49

Results

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

In Table II, the mole concentration of ID_{50}*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 ID_{50} was estimated by the experimental design of 2 doses and 4 repeats.

*5 ID_{50} : 50% inhibitory dose

Table II. ID_{50} of Organophosphoryl Choline Derivatives in Curare-like Action

Signal No.	ID_{50} (M)	Confidence limits	Potency ratio % (for 100 of *d*-tubocurarine)
NP-211 (I)	no action by itself, enhanced ACh action		
NP-212 (I)	no action at $8.85\times10^{-4}M$, ACh-like action at $2.65\times10^{-3}M$		
NP-221 (I)	ACh-like action at $5.42\times10^{-4}M$		
NP-222 (I)	1.52×10^{-4}	$5.82\times10^{-5}\sim3.92\times10^{-4}$	0.68
NP-222 (Br)	9.4×10^{-5}	$2.76\times10^{-5}\sim3.20\times10^{-4}$	1.10
NP-224 (I)	1.07×10^{-6}	$4.89\times10^{-7}\sim2.34\times10^{-6}$	92.2
NP-224 (Br)	1.06×10^{-6}	$4.06\times10^{-7}\sim2.77\times10^{-6}$	97.1
NP-222, 4 (I)	9.00×10^{-6}	$4.05\times10^{-6}\sim2.00\times10^{-5}$	11.4
NP-222, 4 (Br)	1.00×10^{-5}	$4.22\times10^{-6}\sim2.30\times10^{-5}$	10.3
NP-232 (I)	no action by itself, enhanced ACh at $10^{-7}\sim10^{-4}M$		
NP-233 (I)	6.8×10^{-5}	$2.75\times10^{-5}\sim1.69\times10^{-4}$	1.52
NP-242 (I)	2.5×10^{-5}	$7.72\times10^{-6}\sim8.10\times10^{-5}$	4.12
NP-242 (Br)	2.77×10^{-5}	$9.86\times10^{-6}\sim7.78\times10^{-5}$	3.72
NP-243 (I)	7.20×10^{-6}	$2.72\times10^{-6}\sim1.91\times10^{-5}$	14.3
NP-243 (Br)	5.2×10^{-6}	$1.60\times10^{-6}\sim1.69\times10^{-5}$	19.8
NP-244 (I)	6.9×10^{-7}	$2.38\times10^{-7}\sim2.00\times10^{-6}$	149
NP-245 (I)	4.2×10^{-7}	$1.29\times10^{-8}\sim1.37\times10^{-7}$	243
NP-244pC (I)	1.13×10^{-7}	$4.18\times10^{-8}\sim3.05\times10^{-7}$	911
NP-244pB (I)	4.22×10^{-7}	$5.70\times10^{-8}\sim3.12\times10^{-6}$	244
d-Tubocurarine	1.03×10^{-6}	$3.12\times10^{-7}\sim3.40\times10^{-6}$	100

Table III. ID_{50} 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 $5\times10^{-5}M$		
NP-212 (I)	more than $5\times10^{-5}M$		
NP-221 (I)	more than $5\times10^{-5}M$		
NP-222 (I)	1.90×10^{-5}	$8.68\times10^{-6}\sim4.16\times10^{-5}$	0.03
NP-224 (I)	6.43×10^{-6}	$2.47\times10^{-6}\sim1.67\times10^{-5}$	0.10
NP-222, 4 (I)	1.16×10^{-5}	$3.93\times10^{-6}\sim3.42\times10^{-5}$	0.05
NP-232 (I)	ACh-like action at $2.7\times10^{-5}M$		
NP-233 (I)	more than $5\times10^{-5}M$		
NP-242 (I)	2.26×10^{-5}	$8.53\times10^{-6}\sim5.99\times10^{-5}$	0.03
NP-243 (I)	7.70×10^{-6}	$3.52\times10^{-6}\sim1.69\times10^{-5}$	0.07
NP-244 (I)	4.91×10^{-7}	$2.05\times10^{-7}\sim1.18\times10^{-6}$	1.28
NP-245 (I)	6.09×10^{-6}	$2.77\times10^{-6}\sim1.34\times10^{-5}$	0.10
NP-244pC (I)	4.29×10^{-6}	$2.32\times10^{-6}\sim7.71\times10^{-6}$	0.15
NP-244pB (I)	5.25×10^{-6}	$3.04\times10^{-6}\sim9.08\times10^{-6}$	0.12
Atropine	6.29×10^{-9}	$2.47\times10^{-9}\sim1.60\times10^{-8}$	100

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

In Table IV, the mole concentrations of ID_{50} 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, $3\times10^{-5}M$. These potencies were estimated by an experimental design of 2 doses and 4 repeats.

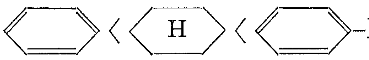
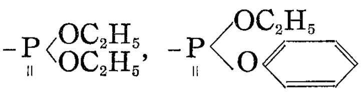
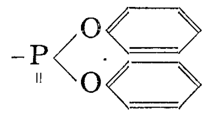
Table IV. ID_{50} of Organophosphoryl Choline Derivatives in Anticholinesterase Action

Signal No.	ID_{50} (*M*)	Confidence limits	Potency ratio % (for 100 of neostigmine)
NP-211 (I)	9.73×10^{-3}	$2.32\times10^{-3}\sim4.09\times10^{-2}$	almost 0
NP-212 (I)	1.25×10^{-3}	$2.02\times10^{-4}\sim7.75\times10^{-3}$	0.01
NP-221 (I)	3.80×10^{-4}	$1.19\times10^{-4}\sim1.22\times10^{-3}$	0.05
NP-222 (I)	3.05×10^{-5}	$4.49\times10^{-6}\sim2.07\times10^{-4}$	0.57
NP-224 (I)	5.11×10^{-6}	$1.25\times10^{-6}\sim2.10\times10^{-5}$	3.39
NP-222, 4 (I)	5.57×10^{-5}	$6.55\times10^{-6}\sim4.73\times10^{-5}$	0.31
NP-232 (I)	1.98×10^{-6}	$4.04\times10^{-7}\sim9.70\times10^{-6}$	8.77
NP-233 (I)	1.86×10^{-6}	$4.05\times10^{-7}\sim1.06\times10^{-5}$	9.26
NP-242 (I)	6.77×10^{-5}	$1.54\times10^{-5}\sim2.98\times10^{-4}$	0.26
NP-243 (I)	1.78×10^{-3}	$3.63\times10^{-4}\sim8.72\times10^{-3}$	0.01
NP-244 (I)	9.49×10^{-5}	$1.51\times10^{-5}\sim5.98\times10^{-4}$	0.18
NP-245 (I)	5.52×10^{-5}	$8.49\times10^{-6}\sim3.59\times10^{-4}$	0.31
NP-244pC (I)	2.38×10^{-5}	$4.87\times10^{-6}\sim1.17\times10^{-4}$	0.73
NP-244pB (I)	9.19×10^{-6}	$9.28\times10^{-7}\sim9.10\times10^{-5}$	1.88
Neostigmine	1.73×10^{-7}		100

Table V. ID_{50} of Curare-like Action of Organophosphoryl Choline in the Presence of Neostigmine

	ID_{50} (*M*)	Confidence limits	Potency ratio
d-Tubocurarine	9.93×10^{-6}	$1.66\times10^{-6}\sim5.96\times10^{-5}$	1
NP-244 (I)	1.39×10^{-6}	$5.32\times10^{-7}\sim3.63\times10^{-6}$	7.14
NP-245 (I)	4.83×10^{-7}	$9.66\times10^{-8}\sim2.42\times10^{-6}$	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 $(RO)_2\underset{\underset{Y}{\|}}{P}\text{-Z-}CH_2\text{-}CH_2\text{-}N^+(CH_3)_3X^-$, the R radical increases the potency of the curare-like action according to the order of $CH_3 < C_2H_5 < CH(CH_3)_2 <$  -X and even in the combination of two different Rs, their order remains that of $-\underset{\|}{P}\langle^{OC_2H_5}_{OC_2H_5}$, , and . 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 cholines, 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 beween 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 Ⅵ. This selective index is represented by the negative logarithmic coefficient of the ratio of ID_{50} of the two actions.

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

Signal No.	Atropine action / Curare action	Anti-ChE action / 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 Ⅵ 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 of curare was inhibited to a greater degree by neostigmine than the action of organophosphoryl choline. It seems possible, therefore, that some of organophosphoryl choline molecules which was prevented combining ChE by the irreversible inhibition of neostigmine was shifted to enhance its anticholinergic action at ACh receptor. Because, the increase rate of the curare-like action of NP-244(I) and NP-245(I) caused by neostigmine are 7.14/1.49 and 20.6/2.43 for *d*-tubocurarine, and then the two values of these rate are corresponding with that of potency 0.18 and 0.31 of anti-ChE *in vitro*. From some of results above mentioned, it may be concluded that ACh receptor is perhaps different from ChE itself.

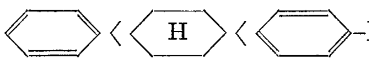
Thanks are given to Mr. Seiich Fukushima and Mr. Tadashi Fujiwara for their assistance in the experimental work.

Summary

To clarify the relationship between anticholinergic and anticholinesterase (anti-ChE) activities on the basis of chemical structure, nineteen organophosphoryl choline derivatives $(RO)_2\text{-}\underset{\overset{\|}{Y}}{P}\text{-Z-}CH_2\text{-}CH_2\text{-}N^+(CH_3)_3X^-$ were synthesized. Curare-like, atropine-like and anticholinesterase actions of these compounds were tested to screen.

The experimental results were as follows :

1) Curare-like and atropine-like actions were both studied by Magnus method, observing their effect on rectus abdominis of frog and on intestines of white mice. Anti-ChE action on enzyme preparation from rabbit serum was tested by using Warburg's manometric method.

2) As to the basic structure of various organophosphoryl choline derivatives, curare-like action was increased in the following order of magnitude; $>\underset{\overset{\|}{O}}{P}\text{-O-} < >\underset{\overset{\|}{O}}{P}\text{-S-} < >\underset{\overset{\|}{S}}{P}\text{-O-} < >\underset{\overset{\|}{S}}{P}\text{-S-}$. The R radicals increased the potency in the following order; $CH_3 < C_2H_5 < CH(CH_3)_2 <$  -X and as for quaternary ammonium salts of derivatives, bromides were more potent than iodides. From these compounds it was found that four derivatives of NP-244(I), NP-245(I), NP-244pC(I) and NP244pB(I) was more potent than curare.

3) On the atropine-like action of organophosphoryl choline derivatives, the relation between their structure and potency was found to have the same tendency as in the case of curare-like action, but all of these derivative were weaker than that of atropine.

4) On the anti-ChE action of organophosphoryl choline, the basic structures increased its potency in the following order; $>\underset{\overset{\|}{O}}{P}\text{-O-} < >\underset{\overset{\|}{S}}{P}\text{-S-} < >\underset{\overset{\|}{S}}{P}\text{-O-} < >\underset{\overset{\|}{O}}{P}\text{-S-}$, but the change in R radicals did not always increase the potency of their curare-like action. All of these derivatives were weaker than neostigmine.

5) The relationship between curare-like and atropine-like actions and between curare-like and anti-ChE actions were discussed with the selective indexes calculated from experimental results. Some of thionothiol-phosphate have both curare-like and ChE activity, and its curare-like action is little antagonized by neostigmine. From these facts, it was suggested that acetylcholine receptor was perhaps different from ChE.

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