91. Takeo Ueda, Shigeshi Toyoshima, Kiyoshi Takahashi*, Masasuke Ose, Mitsuharu Taniguchi, and Hiroshi Tatsumi**: Researches on Chemotherapeutic Drugs against Viruses. XV.[1] Antiviral Effects of Carbamyloxy-pyridinium and -quinolinium Anionides.

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Although great advances have been made in the treatment of infectious deseases caused by bacteria and rickettsia, there has not been found a drug capable of preventing viral infections of the host, and also of exerting effects on viruses themselves.

It may be said that one possibility in chemotherapy of viral infection should be based on whether viruses possess partial enzymatic systems or not. If such characteristically essential systems could be found in viruses, they might be attacked by antagonistic substances. The possibility that viruses might actually possess unique enzymatic systems has been found in studies on the phenomenon of hemagglutination by the influenza virus.

Hirst *et al.*[2] demonstrated the ability of the influenza virus to bring about agglutination of red blood cells of chicks and postulated the enzymatic nature of this reaction between the virus and red blood cells. Foster[3] suggested that the enzyme in the influenza virus might be cholinesterase. Recently, Toyoshima[4] demonstrated that a similar enzyme should exist in this virus by showing the inhibitive action of cholinesterase-antagonists, such as organic arsenicals, mercury compounds, and prostigmine.

These findings led us to postulate that the influenza virus might be affected by antagonistic substances against the cholinesterase. Consequently several compounds of both carbamyloxy-pyridinium and -quinolinium series were synthesized and their activities against the influenza virus examined.

This paper describes the chemotherapeutic effects of various carbamyloxy-pyridinium and -quinolinium compounds against the influenza virus.

Materials and Methods

The aqueous solutions of fifteen compounds of the carbamyloxypyridinium and -quinolinium series were employed. The PR-8 strain of influenza A virus was employed, which was kindly given to us by Dr. T. Fukai, Institute of Bacteriology, Osaka University.

For the purpose of examining the compounds, the embryonated egg method, the *in vivo* test-method with mice, and the method of estimation of cholinesterase activity were employed.

Results

1) Estimation of Cholinesterase Activity—Fifteen compounds shown in Table I were examined. The estimation of cholinesterase activity was carried out according to the titration method by Hall and Lucas[5]. Rabbit serum was employed as enzyme material.

One cc. of serum was mixed with 1 cc. of diluted solution of each compound in concentration of 1/2,000~1/2,000,000, and after 20 minutes, the mixture was titrated by using a Beckman pH-meter. Results are given in Table II by activity rate(%).

It may be said from Table II that all compounds except benzopyridinium bromide (Stigmonene Bromide) (P-1) exerted weak antagonistic effect against cholinesterase.

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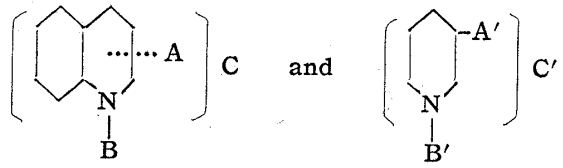
1) Part XIV: This Bulletin, 1, 371(1953).
2) Hirst: Science, 94, 22(1941).
3) Foster, Floyd: Proc. Soc. Exptl. Biol. Med., 20, 66(1947).
4) S. Toyoshima: Papers read before the Annual Meeting of the Pharmaceutical Society of Japan (April 30, 1952).
5) Hall, Lucas: J. Pharmacol., 59, 34(1939).

TABLE I. Compounds employed for the Tests

General formulae:  and

No.	A	B	C
Q-1	$-OC-N(CH_3)_2$ (8)	$-CH_2-C_6H_5$	Br
Q-2	$-OC-N(CH_3)_2$ (8)	$-CH_2-CH_2-NH-C-C_6H_5$ (O)	Br
Q-3	$-OC-NH-C_6H_5$ (8)	$-CH_2-C_6H_5$	Br
Q-4	$-OC-NH-C_6H_5$ (8)	$-CH_2-CH_2-NH-C-C_6H_5$ (O)	Br
Q-5	$-OC-N(CH_3)_2$ (3)	$-CH_2-C_6H_5$	Br
Q-6	$-OC-N(CH_3)_2$ (3)	$-CH_2-CH_2-NH-C-C_6H_5$ (O)	Br
Q-7	$-OC-N(CH_3)_2$ (4) $-CH_3$ (2)	$-CH_2-CH_2-NH-C-C_6H_5$ (O)	Br
Q-8	H	$-CH_2-C_6H_5$	Br

No.	A′	B′	C′
P-1	$-OC-N(CH_3)_2$	$-CH_2-C_6H_5$	Br
P-2	$-OC-N(CH_3)_2$	$-CH_2-CH_2-NH-C-C_6H_5$ (O)	Br
P-3	$-OC-N(CH_3)_2$	$-CH_2-O-C_{12}H_{25}$	Cl
P-4	$-OC-N(CH_3)_2$	$-C_{12}H_{25}$	Br
P-5	$-OC-N(CH_3)_2$	$-C_{18}H_{37}$	Br
P-6	$-OC-NH-C_6H_5$	$-CH_2-C_6H_5$	Br
P-7	$-OC-NH-C_6H_5$	$-CH_2-CH_2-NH-C-C_6H_5$ (O)	Br

2) Tests in Embryonated Eggs —The PR-8 strain was employed for the tests. 0.5 cc. of each virus dilution was inoculated into the allantois of embryonated egg, which had previously been incubated at 37° for 11 days. One hour later, 0.1 cc. of each solution (1 mg. in 1 cc. of distilled water) was injected into the egg. The presence of demonstrable hemagglutin-

TABLE II. Antagonistic Activity of the Compounds against Cholinesterase

Concn. / Compd.	1/2,000	1/20,000	1/200,000	1/2,000,000
Q-1	44.9%	50.3%	65.5%	79.0%
Q-2	—	45.5	64.4	76.0
Q-3	—	50.2	71.0	76.0
Q-4	—	40.6	61.3	68.0

ation in the allantoic fluid collected from the egg after 48 hours' incubation at 37°, was used as the criterion of the infection. Results are given in Table III, in which (+) represents the growth of the virus and (−), the inhibition of the growth of the virus.

Table III shows that Q-2, Q-4, P-1, P-5, P-6, and P-7 showed remarkable activities on the virus among the compounds employed.

Q-5	44.1	55.4	70.0	77.0
Q-6	—	—	50.0	80.0
Q-7	—	—	50.0	78.0
Q-8	—	—	60.5	67.0
P-1	3.0	10.1	40.2	60.0
P-2	45.1	58.1	72.3	90.2
P-3	50.5	67.4	81.5	95.8
P-4	51.5	72.7	78.7	94.0
P-5	37.8	46.8	52.7	75.4
P-6	30.1	40.1	62.1	73.2
P-7	32.7	46.1	58.5	72.1

TABLE III. Antiviral Activities in Embryonated Egg

Virus diln. / Compd.	10	20	40	80	160	320	640	1280	2560	5120
Q-1	+	+	+	+	+	+	+	—	—	—
Q-2	—	—	—	—	—	—	—	—	—	—
Q-3	+	+	+	+	+	+	+	—	—	—
Q-4	—	—	—	—	—	—	—	—	—	—
Q-5	+	+	+	+	+	+	—	—	—	—
Q-6	+	+	+	+	+	+	+	—	—	—
Q-7	+	+	+	+	+	+	+	—	—	—
Q-8	+	+	+	+	+	+	+	—	—	—
P-1	—	—	—	—	—	—	—	—	—	—
P-2	+	+	+	+	+	+	+	—	—	—
P-3	+	+	+	+	+	+	+	—	—	—
P-4	+	+	+	+	+	+	+	—	—	—
P-5	—	—	—	—	—	—	—	—	—	—
P-6	—	—	—	—	—	—	—	—	—	—
P-7	—	—	—	—	—	—	—	—	—	—
Control	+	+	+	+	+	+	+	+	+	—

3) Tests *in vivo*—Mouse lungs infected with the virus were employed for the tests. Dilution of infective mice lungs was prepared with unactivated serum-Lush's solution, and 0.02 cc. of 10^{-3} dilution was inoculated by the intranasal inoculation technique under ether anesthesia, into groups of 20 mice each (ca. 10 g. body weight). After various intervals aqueous solutions of each compound were injected intravenously into the above mice. These treated mice were observed during seven days, and the chemotherapeutic effects of those compounds were determined by the mortality of the mice suffering from the virus. The results are given in Table IV by survival ratio.

TABLE IV. Antiviral Effects *in vivo*

Dose / Administ. time (hrs.) / Compd.	1×10 mg./kg. 0	4×5 mg./kg. 0-3-12-24	4×5 mg./kg. 3-12-24-36	3×5 mg./kg. 12-24-48	Untreated Control
Q-1	0/7	0/3	1/4	1/16	0/10
Q-2	3/17	3/13	—	7/19	0/8
Q-3	0/17	1/9	—	2/14	0/8
Q-4	4/16	7/12	6/12	8/18	0/8
Q-5	1/17	1/9	2/18	1/16	0/10
Q-6	1/17	3/14	—	1/17	0/8
Q-7	1/18	0/16	—	2/15	0/8
Q-8	2/13	1/17	2/15	2/17	0/10
P-1	10/16	12/17	10/18	9/19	0/10
P-2	1/17	3/14	—	6/18	0/10
P-3	2/19	2/15	—	3/15	0/10
P-4	3/13	1/11	—	2/14	0/8
P-5	3/8	4/14	—	4/11	0/8
P-6	4/8	7/16	—	6/17	0/8
P-7	3/7	7/18	—	7/16	0/8

The numerator represents the number of mice that survived, the denominator, the difference of the total number injected and the number of uninfected mortal mice.

Table IV shows that Q-2, Q-4, P-1, P-5, P-6, and P-7 showed remarkable effects *in vivo* on the virus within 12 hours after the inoculation.

Discussion and Conclusion

It is noted that chemoprophylactic and chemotherapeutic effects with the influenza virus are scarcely observed. Though several substances were found to be effective against the influenza virus in using various test methods, none of them exerted enough remarkable *in vivo* effects to prompt clinical testing.

On the contrary, the compounds of the carbamyloxy-pyridinium and quinolinium series were synthesized and their effects on the influenza virus examined according to the postulation that the virus possessing cholinesterase might be prohibited by anticholinesterase substances. The observations with these compounds, as described in the experimental part, show that these compounds were more or less effective against the virus. Among these compounds, Q-2, Q-4, P-1, P-5, P-6, and P-7 were observed to possess remarkable effects approximately equal to the anticholinesterase drug, Prostigmine[6]. The virulence of the PR-8 strain possessing LD_{50} $10^{-4.5}$, decreased up to ca. 10^{-2} with each of the above six compounds. Moreover, it should be noted that these compounds were invariably effective 12 hours after inoculation of the virus into host (coinciding with the period of resynthesis and multiplication of the virus in host).

Both Q-2 and Q-4 were more beneficial in toxicity, because these two compounds possessed comparatively lower toxicity of LD_{50} 100 mg./kg., compared with Prostigmine (LD_{50} ca. 0.2 mg./kg.) and the compounds of the pyridinium series (LD_{50} 30 mg./kg.).

At the present stage it is not timely to discuss the mode of action of these compounds on the virus. However, the antiviral activities of these compounds might be supposed to be caused partially by their antichlolinesterase properties, though their *in vivo* effects were not strictly parallel to their anticholinesterase activities. The mode of action of these compounds will be discussed in a medical journal in detail. It may be concluded that carbamyloxyquinolinium compounds are of interest for clinical use.

Further work on this problem is in progress.

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Summary

(1) Fifteen compounds of the carbamyloxy-pyridinium and -quinolinium series were examined as to their anticholinesterase activities and *in vivo* effects.

(2) Two compounds of the carbamyloxypyridinium series and four compounds of the carbamyloxyquinolinium series exerted remarkable effects *in vivo*.

(3) It is assumed that the antiviral activities of these compounds might be caused partially by their anticholinesterase activities.

(4) Compounds of the carbamyloxyquinolinium series seem to be of promise for clinical use.

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6) S. Toyoshima: Papers read at the Annual Meeting of the Pharmaceutical Society of Japan (April 30, 1952).