Inhibitory Effect of Cyanine- and Styryl-Dyes upon Cholinesterase. I.

By Eiji HASE, Yoshihisa MIZUNO and Masujiro KATAYANAGI.

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During the past several years it has repeatedly been reported that certain cyanine- and styryl-dyes, usually known as photosensitizers,[1] have some therapeutic effect against certain kind of diseases.[2] The mechanism

(1) T Ogata, "Photosensitive Dyes" ("Kankō-shikiso" in Japanese), Sankaidō-shoten, Tokyo, 1941.

(2) A. D. Welch, L. Peters, E. Beuding, A. Valk and A. Higashi, *Science*, **105** (1947), 486; L. G. S. Brooker and L. A. Swett, *Science*, **105** (1947), 496.

of this chemotherapeutic action remains as yet wholly unknown. Not only from the pharmacological point of view, but also from the purely enzymological view-point, it may be worth while to investigate the action of these substances which have the peculiar physico-chemical properties upon the activities of different enzymes.

Brooker and Swett[2] have reported that cyanine- and styryl-dyes have no appreciable effect upon various oxidative enzymes studied. On the other hand, Hino and his coworkers[3] have shown that some of these dyes exhibit strong inhibiting action upon catalase. In the present study, it is shown that certain substances belonging to the same category exert remarkable suppressing effect upon the activity of cholinesterase. Whether this effect has anything to do with the chemotherapeutic action reported or not is the problem to be worked out in the future.

Experimental Method.

The activity of cholinesterase was measured by the potentiometric titration method after Alles and Hawes[4] with a slight modification for our experimental purpose. The dyes were dissolved in one per cent ethyl alcohol solution.* The mixture of enzyme and dye was regulated to *p*H 7.5 and left in a thermostat at 30°C for 10 min. After the addition of the substrate, the acid liberated by hydrolysis of ester was constantly neutralized with 0.04 N NaOH and its volume consumed was recorded against time. The estimation of the reaction rate was made on the initial part of the alkali-time curve thus obtained. The concentration of the substrate used was sufficiently high so that the rate of hydrolysis remained practically constant during the course of the experiment (10 min.).

Results.

Inhibition of the cholinesterase activity in horse serum by certain cyanine- and styryl-dyes. The inhibitory effects of about one hundred of cyanine- and styryl-dyes were examined. It was found that almost all of them show more or less strong inhibitory action upon cholinesterase activity in horse serum. Several examples of these dyes which exhibit most strong inhibitory effects are shown in Table 1. Their efficacies are compared in terms of concentration causing 50 per cent inhibition with those of substances which have thus far been known as strong inhibitors of cholinesterase.

It may be seen that the action of dyes is by far stronger than most of the substance listed, although it is less than one hundredth of the activity of eserine.

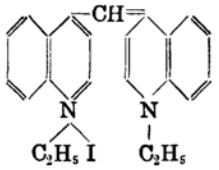
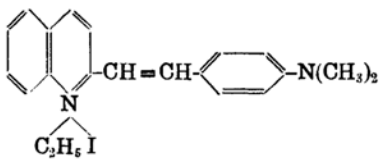
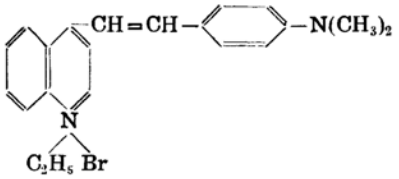
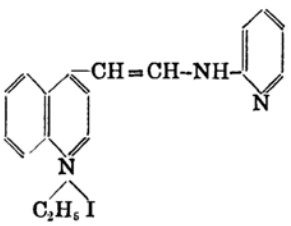
(3) S. Hino, Y. Ogura, M. Fujita and K. Shibata, Unpublished paper read before the Chem. Soc. Jap. in 1948.

(4) G. A. Alles and R. C. Hawes, *J. Biol. Chem.*, **133** (1940), 377.

* It was ascertained that ethyl alcohol had no effect upon cholinesterase under these experimental conditions.

Table 1.

Inhibitory effects of some cyanine- and styryl-dyes upon cholinesterase in horse serum.

Inhibitor		Concentration of the inhibitor causing 50% inhibition (mol/l.).
Cyanine-dyes	 1, 1′-Diethyl-4, 4′-monomethine-quinocyanine iodide	$10^{-5.4}$
	$C_{30}H_{39}S_2N_2I$	$10^{-5.5}$
Styryl-dyes	 *p*-Dimethylamino-benzyliden-quinaldine ethiodide	$10^{-5.6}$
	 *p*-Dimethylamino-benzyliden-lepidine ethobromide	$10^{-5.4}$
	 4-(α-Pyridylamino)-ethenyl-quinoline ethiodide	$10^{-5.4}$
Other substances	Eserine salicylate	$10^{-7.9}$
	Strychnine sulphate	$10^{-4.6}$
	Quinine hydrochloride	$10^{-4.1}$
	Atropine sulphate	$10^{-2.6}$

All these measurements were carried out under the same experimental conditions; *p*H of the reaction mixture, 7.5; concentration of the substrate (acetylcholine chloride), 0.02 mol/l.; the temperature, 30°C.

Effects upon the "specific" and "non-specific" cholinesterase. Recently it has been found[5] that there are two types of cholinesterase, the "specific" and the "non-specific". Using one of the most active cyanine dyes found in the previous experiment, the susceptibility of the two different types of cholinesterase was compared. As may be seen in Table 2 the non-specific cholinesterase seems to be more strongly inhibited than the specific one. Since, however, the preparation of the specific cholinesterase used in this experiment was not sufficiently homogeneous, the results obtained do not allow us to conclude definitely and quantitatively the difference in the susceptibilities of the two cholinesterases.

Table 2.

Effects of some cyanine- and styryl-dyes upon various esterases.

Dye	Enzyme	Source of enzyme	Substrate	Concentration of the dye causing 50% inhibition of enzyme activity (mol/l.)
1, 1′-Diethyl-4, 4′-mono-methine-quino-cyanine iodide	Non-specific cholinesterase	Horse serum	Acetylcholine chloride (0.02 mol/l.)*; *p*H, 7.5; 30°C	$10^{-5.4}$
,,	Specific cholinesterase	Homogenate of horse brain	Acetylcholine chloride (0.002 mol/l.)*; *p*H, 7.5; 30°C	$10^{-4.6}$～$10^{-4.8}$
,,	Esterase	Horse serum	Methylbutyrate and triacetin at various concentrations; *p*H, 7.5; 30°C	Slight activation
Various cyanine- and styryl-dyes	Esterase	Pig liver extract	Methylbutyrate (0.1 mol/l.); *p*H, 7.5; 30°C	No measurable effect

* Concentration of substrate applied was nearly saturating for the reaction velocity observed in each case.

Effects upon other esterases. Effects of cyanine- and styryl-dyes upon the esterase in horse serum were examined, methyl butyrate and triacetin being used as the substrate. It was found that some of the dyes examined increase slightly the activity of this enzyme and others have no measurable effects. It was further ascertained that the dyes have no appreciable effects upon the activity of liver esterase (Table 2).

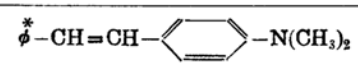
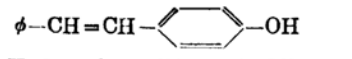
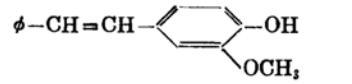
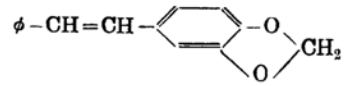
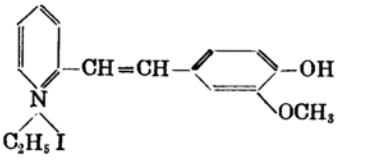
At any rate, it can be concluded that the inhibitory effect of the dyes is quite specific to the cholinesterase, whether "specific" or "non-specific".

(5) A. M. Wynne, *Ann. Rev. Biochem.*, **15** (1946), 63.

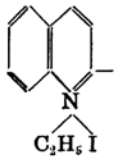
Relationship between the chemical constitution and the inhibitory effect. Comparison of efficacy of structurally related styryl-dyes shows us that there exists certain relationship between structure and inhibitory effect (see Table 3). It can be seen from the table that the dimethylamino radical, attached to benzene ring in the molecule, has the most favorable effect upon the inhibitory action of the dye.

It is another interesting problem that vanillylidenpicolin ethiodide, which contains a pyridine nucleus, has no appreciable effect upon cholinesterase, while vanillylidenquinaldine ethiodide, possessing a quinoline nucleus, exhibits a considerable inhibitory action under the same experimental conditions.

Table 3. Relationship between the chemical constitution of the related styryl-dyes and their inhibitory effects upon the cholinesterase in horse serum.

	Chemical structure of dye	Concentration of the dye causing 50% inhibition (mol/1.)**
I	 *p*-Dimethylamino-benzyliden-quinaldine ethiodide	$10^{-5.6}$
II	 *p*-Hydroxybenzyliden-quinaldine ethiodide	$10^{-5.0}$
III	 Vanillyliden-quinaldine ethiodide	$10^{-4.8}$
IV	 Piperonyliden-quinaldine ethiodide	$10^{-4.7}$
V	 Vanillyliden-picoline ethiodide	No appreciable effect even at the concentration of $10^{-4.0}$ mol/1.

* The symbol ϕ represents the quinaldine ethiodide nucleus



** Experimental condition; *p*H, 7.5; temperature, 30°C; concentration of substrate (acetylcholine chloride), 0.02 mol/1.

Summary.

(1) It was found that a number of cyanine- and styryl-dyes has a strong inhibitory action upon cholinesterase.

(2) Both the specific and non-specific cholinesterase are inhibited by the subtances, the latter being probably more susceptible than the former.

(3) The substances have no appreciable effects upon the liver esterase. With the esterase in horse serum, even a slight acceleration of the activity was observed under the influence of some dyes investigated.

(4) For some related members of styryl-dyes, a certain relationship between chemical constitution and their inhibitory effects upon cholinesterase could be found.

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