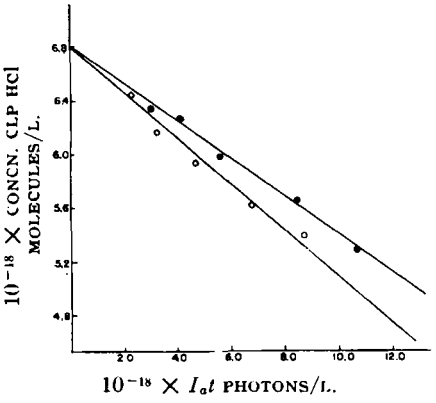


Fig. 6.—A plot of concentration of CLP HCl *vs.* number of photons/liter absorbed showing the zero-order dependency of the photoreaction. Key: ○, under a normal atmosphere, slope = 0.18; ●, under an oxygen-free atmosphere, slope = 0.14.

total photons absorbed per milliliter, $I_a t$, is included (Fig. 6).

## SUMMARY

The presence of a semiquinone free radical as an intermediate in the ultraviolet photolysis of CLP HCl was demonstrated. Disproportionation of this free radical intermediate yields the photolabile CLP-O HCl, which apparently is the precursor to phenolic and other unidentified degradation products formed on irradiation.

Photodegradation of CLP HCl seemed to be more dependent on the presence of water than dissolved oxygen, and it would not be expected that elimination of oxygen alone would have a marked effect on the degradation of ultraviolet irradiated solutions of CLP HCl at 253.5 mμ.

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# Alterations in the Activity of Pentothal, Phenobarbital, Pentylenetetrazol, and Strychnine by Cholinesterase Inhibitors

By VERNON A. GREEN

**Mice pretreated with anticholinesterases showed enhanced stimulation in the presence of pentylenetetrazol and strychnine, a decreased lag period with anesthetic doses of phenobarbital, and a prolongation of anesthesia with pentothal. An attempt was made to correlate these changes in response with the depression of acetylcholine hydrolyzing enzymes.**

BECAUSE THE brain has higher concentrations of acetylcholinesterase than other areas of the body (1), attempts to potentiate drug action through cholinesterase inhibition should be accomplished more readily with agents which primarily affect the central nervous system.

The findings of potentiation of the rate of diffusion of acid fuchsin (2) in frog and dog (3), trypan red (4) in dog, and the potentiation of morphine (5) in cats, streptomycin on *Escherichia coli* (6) and rats (7), and barbital (8) in mice by neostigmine or physostigmine were indications that possibly the activity of other drugs could be potentiated.

In this study, physostigmine, neostigmine, and diisopropylfluorophosphate (DFP) were used as prior medication in mice in an attempt to potentiate the action of some central nervous system stimulants and central nervous system depressants. The basic criteria for the determination of potentiation were the ability of cholinesterase inhibitors to render a subconvulsive dose of a central nervous system stimulant convulsive (9)—

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TABLE I.—DETERMINATION OF CHOLINESTERASE INHIBITION IN MOUSE BRAIN AND BLOOD

| Group | Units[a] Esterase Activity | | Depression, % | |
|---|---|---|---|---|
| | Blood | Brain | Blood | Brain |
| Control | 0.558 | 0.846 | 0 | 0 |
| Physostigmine | 0.495 | 0.781 | 11.3 | 7.6 |
| Neostigmine | 0.421 | 0.700 | 24.5 | 17.0 |
| DFP | 0.510 | 0.822 | 8.5 | 2.9 |

[a] A unit of cholinesterase for this study is defined as the number of milliliters of 0.1 *M* acetic acid derived from 3 ml. of 0.1 *M* acetylcholine bromide solution by the cholinesterase activity in 1 Gm. of mouse brain or 1 ml. of whole blood in 10 minutes at a constant pH of 8.

and in the case of CNS depressants—to decrease the anesthetic lag, to prolong the anesthetic time of a given dose, or to increase the anesthetic activity of a smaller dose.

## EXPERIMENTAL AND DISCUSSION

### Determination of Cholinesterase Inhibition

Eighty mice (28 to 40 Gm., no regard to sex or age) were divided into four groups of 20 each. Group *A* was injected with 0.0004 mg./Gm. physostigmine salicylate; group *B* received 0.0004 mg./Gm. neostigmine; group *C* received DFP (2 mg./Kg.); and group *D* was used as the control. The animals were etherized and then decapitated 15 minutes after the injection of the cholinesterase inhibitors. The brain and blood were removed for the determination of cholinesterase content.

The degree of cholinesterase inhibition was determined and reported in units per gram of brain and milliliters of whole blood as shown in Table I.

### Strychnine Potentiation

The possible potentiation of strychnine sulfate (0.00025 mg./Gm.) by cholinesterase inhibitors was studied on mice with no regard to age or sex. The cholinesterase inhibitor was administered prior to the strychnine; any increase in the stimulating activity above that of the 0.00025 mg./Gm. controls was interpreted as potentiation.

Twenty mice injected subcutaneously with 0.0004 mg./Gm. of physostigmine salicylate had convulsions and died in an average of 40.35 seconds following 0.00025 mg./Gm. of strychnine sulfate intravenously (Fig. 1), but none of the controls showed visible signs of stimulation (Fig. 1).

Twenty mice injected subcutaneously with neostigmine methylsulfate (0.0004 mg./Gm.) 15 minutes before strychnine sulfate (0.00025 mg./Gm.) had convulsions and died (Fig. 1), while the same number of mice with 2 mg./Kg. of DFP 2.5 hours prior to strychnine showed only convulsions (Fig. 1).

Since cholinesterase inhibitors cause vasodilation either directly or indirectly through the accumulation of acetylcholine, an attempt was made in this study to produce vasodilation by the administration of acetylcholine. Of 23 mice injected intravenously with 0.05 mg./Kg. of acetylcholine bromide plus strychnine sulfate, all showed convulsive seizures of short duration with only one death (Fig. 1).

To study the effects of possible vasodilation on potentiation of strychnine further, attempts were made to prevent vasodilation with a parasympatholytic drug. Twenty mice were given the cholinesterase inhibitors with atropine sulfate (0.1 mg./Gm.) 15 minutes prior to the intravenous strychnine, with no appreciable change in the stimulating activity above or below that of the animals receiving inhibitor and strychnine alone (Fig. 1). Physostigmine prior to strychnine and acetylcholine combined showed erratic results (Fig. 1).

Schweitzer (10) reported that neostigmine and acetylcholine depressed spinal reflexes, thereby inhibiting strychnine induced convulsions. The opposite action was found in this study since a great potentiation of a subconvulsive dose of strychnine was noted by neostigmine and a small potentiation by acetylcholine. It is true, however, that large amounts of acetylcholine will decrease nerve transmission since it will keep nerve endings depolarized. Therefore, large doses of neostigmine or acetylcholine could depress spinal reflexes, but with the doses used in this study, there was potentiation of strychnine activity instead of depression.

### Pentylenetetrazol Potentiation

The use of strychnine as a drug is almost purely academic, and since Nachmansohn (12) has reported strychnine as a cholinesterase inhibitor *in vitro*, it was decided to attempt potentiation of another

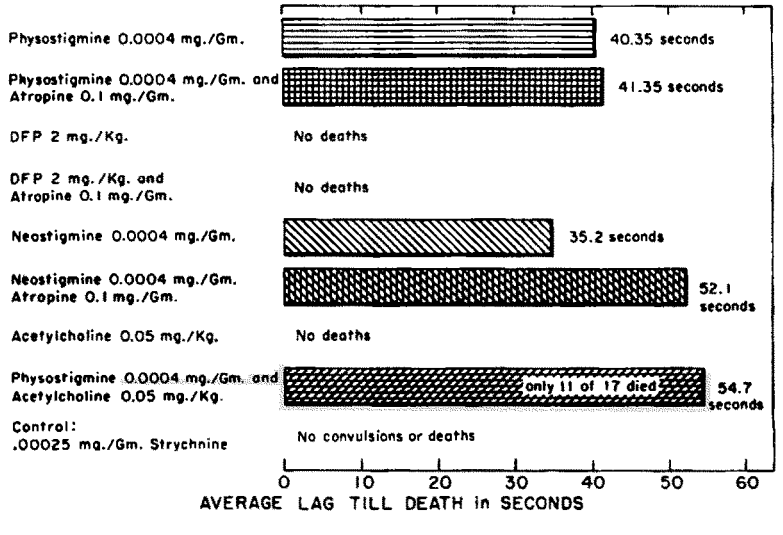



Fig. 1. — Effect of cholinesterase inhibitors, atropine, and acetylcholine on CNS stimulating activity of strychnine (0.00025 mg./Gm.) intravenously in mice.

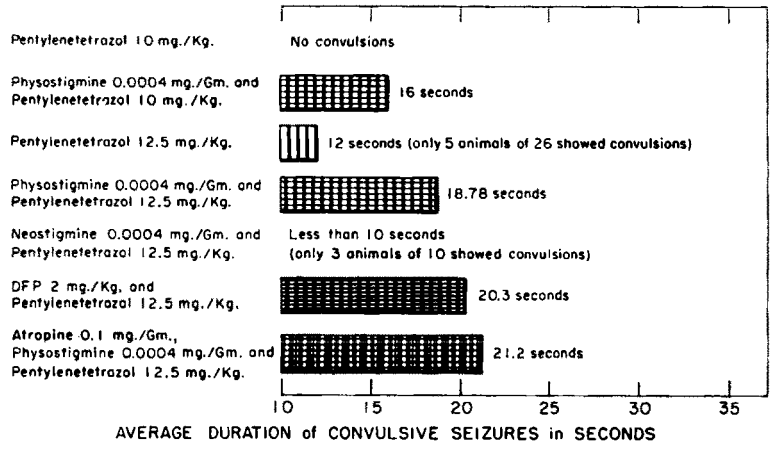



Fig. 2.— Effect of cholinesterase inhibitors and atropine on activity of pentylenetetrazol in mice.

CNS stimulant—one used medicinally today that is not thought to be a cholinesterase inhibitor.

Pentylenetetrazol was given to mice in doses of 10, 12.5, and 15 mg./Kg. intravenously to determine a minimal convulsive dose. It was found that 10 mg./Kg. was subconvulsive in all mice used in this study (Fig. 2). Five of 26 mice showed convulsive seizures of very short duration with 12.5 mg./Kg.; therefore, these two dose levels were selected for this study.

Nineteen of 20 mice receiving 0.0004 mg./Gm. of physostigmine 15 minutes prior to pentylenetetrazol intravenously, 10 mg./Kg., showed convulsive seizures of an average duration of 15.8 seconds (Fig. 2), while there were no convulsions in the 10 mg./Kg. controls. On using 12.5 mg./Kg. of pentylenetetrazol 15 minutes after physostigmine, all 28 mice showed convulsive seizures compared to five convulsive seizures in 26 mice used as 12.5 mg./Kg. controls (Fig. 2), but no deaths occurred.

Neostigmine, 0.0004 mg./Gm. of mouse, 15 minutes prior to pentylenetetrazol, 12.5 mg./Kg., gave convulsions in all 20 mice tried (Fig. 2), while DFP (2 mg./Kg.) 2.5 hours prior to pentylenetetrazol gave convulsions in only six mice of 20 tried and the seizures were like those of pentylenetetrazol alone, an average of 7 seconds.

In an attempt to rule out a vasodilating action of physostigmine and neostigmine, atropine sulfate (0.1 mg./Gm. of mouse) was administered subcutaneously with the cholinesterase inhibitor with no appreciable change in the percentage of seizures or duration of seizures above or below those occurring in mice which received only inhibitor and CNS stimulant (Fig. 2).

### Alteration of Action of Phenobarbital Sodium

Greig and Mayberry (8) reported a decrease in the anesthetic lag time of barbital sodium in mice receiving prior medication with physostigmine. In addition, the above investigators reported an increase in the brain tissue level of the barbital following prior medication with physostigmine. More recently, in 1958, Rosenberg (11) found an increase in the sleeping time of hexobarbital following prior medication with some of the organic phosphates that are cholinesterase inhibitors.

Since the above-mentioned investigations reported potentiation of barbital and hexobarbital, this investigator attempted to study the effects of physostigmine, neostigmine, and DFP on the anesthetic lag time of phenobarbital sodium in mice, with no consideration of age, weight, or sex.

A dose of 160 mg./Kg. of phenobarbital sodium was given to 50 mice intravenously, and the average lag time from administration until onset, loss of righting reflex, was determined as 1147 seconds (Fig. 3). This average lag time was used as the

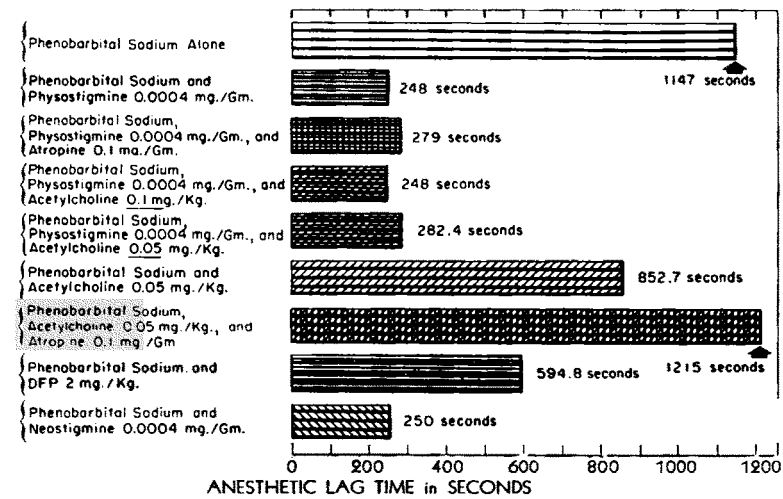



Fig. 3.—Effect of physostigmine, neostigmine, DFP, atropine, and acetylcholine on anesthetic lag of intravenous phenobarbital sodium (160 mg./Kg.) in mice.

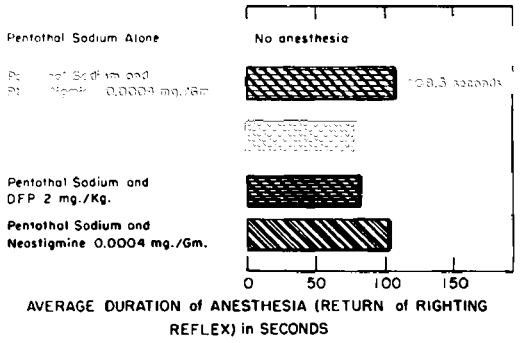



Fig. 4.—Effect of cholinesterase inhibitors and atropine on duration of anesthesia produced by 10 mg./Kg. of pentothal sodium intravenously in mice.

basis of comparison to determine any decrease in the lag time until onset of action that might be caused by prior medication with cholinesterase inhibitors.

Thirty mice receiving physostigmine salicylate (0.0004 mg./Gm.) subcutaneously 15 minutes prior to phenobarbital sodium (160 mg./Kg. intravenously) showed an average lag time until onset of 258.4 seconds (Fig. 3). This represents about a 75% decrease from the values for the controls receiving 160 mg./Kg. of phenobarbital alone.

DFP (2 mg./Kg.) subcutaneously as prior medication 2.5 hours previous to intravenous phenobarbital (160 mg./Kg.) gave an average lag time of 594.8 seconds (Fig. 3), while neostigmine (0.0004 mg./Gm.) subcutaneously 15 minutes prior to phenobarbital, gave an average lag time of 250.7 seconds (Fig. 3). This represents a 50% decrease for DFP and a 75% decrease for neostigmine below the lag time of the controls.

Other investigators reporting potentiation of barbiturate-induced sleeping time and decreased lags did not attempt to preclude the effects of vasodilation as a possible cause of the potentiation. It was decided that in this study the investigator would not only use a parasympatholytic in an attempt to block the acetylcholine vasodilation, but would also try to potentiate the barbiturate depression by acetylcholine-induced vasodilation. If the potentiation were the result of increased tissue uptake due to dilation of vessels, atropine should block it, and acetylcholine should produce similar potentiations.

Twenty mice receiving atropine sulfate (0.1 mg./Gm.) and physostigmine salicylate (0.0004 mg./Gm.) 15 minutes prior to phenobarbital gave an average lag time of 279 seconds. Twenty-four animals receiving physostigmine 15 minutes prior to acetylcholine bromide (0.05 mg./Kg.) plus phenobarbital sodium showed an average lag time of 282 seconds, while 20 animals receiving physostigmine 15 minutes prior to 0.1 mg./Kg. acetylcholine plus phenobarbital showed an average lag time of 248 seconds.

Acetylcholine bromide (0.05 mg./Kg.) plus 160 mg./Kg. of phenobarbital sodium intravenously showed an average lag time of 852.7 seconds (Fig. 3); and atropine sulfate (0.1 mg./Gm.) 15 minutes prior to acetylcholine (0.05 mg./Kg.) plus phenobarbital gave an average lag time of 1215 seconds.

These findings indicate that some method of potentiation other than vasodilation is involved in the potentiating action of these anticholinesterases. However, a vasodilating action could be involved in the mild potentiating action of the acetylcholine.

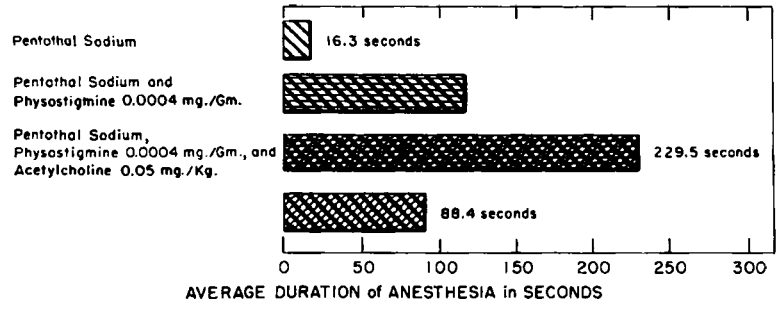
### Pentothal Sodium Potentiation

Pentothal sodium was administered to mice in the amounts of 10 mg./Kg., 15 mg./Kg., and 35 mg./Kg. intravenously, and the anesthetic times or time lapses from loss of righting reflex until righting reflexes were regained were recorded. The time lapses from onset until recovery of walking ability was regained were also noted for comparison with controls.

Twenty mice injected with physostigmine salicylate 15 minutes prior to 10 mg./Kg. pentothal sodium gave an average anesthetic time of 108.5 seconds until recovery of righting reflex and 206.4 seconds until walking ability was regained (Fig. 4). Atropine sulfate (0.1 mg./Gm.) administered 5 minutes before the physostigmine in other mice (1) decreased the average anesthetic time to 79.1 seconds and the regaining of walking ability to 122 seconds. These average anesthetic times were in direct contrast to the controls, since in 20 control mice, 10 mg./Kg. of pentothal sodium produced no loss of righting reflex.

DFP and neostigmine produced average anesthetic times with 10 mg./Kg. of pentothal sodium of 82.7 and 102.2 seconds, respectively, while the time lags until walking was regained were 196.1 and 212.6 seconds.

The 15 mg./Kg. pentothal controls showed an average anesthetic time of 16.3 seconds and 30 seconds before walking ability was regained, but in 20 mice premedicated with physostigmine, the average anesthetic time was 116.2 seconds and 205 seconds until walking was regained (Fig. 5). When acetylcholine bromide (0.05 mg./Kg.) was given with 15 mg./Kg. pentothal sodium to physostigminized animals, the average anesthetic time increased to 229.5 seconds, and the recovery of walking ability to 326 seconds. Acetylcholine with pentothal sodium and no prior medication showed



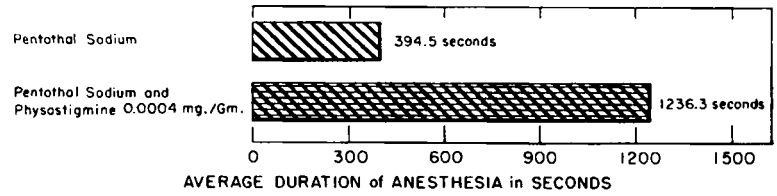



Fig. 6.—Effect of physostigmine on duration of anesthesia produced by 35 mg./Kg. of pentothal sodium intravenously in mice.

an average anesthetic time of 88.4 seconds and no prior medication showed an average anesthetic time of 88.4 seconds and 110.7 seconds until walking was recovered. These values were approximately the same as those for animals receiving DFP and pentothal.

Twenty physostigmine treated animals receiving 35 mg./Kg. of pentothal sodium gave an average anesthetic time of 1236.3 seconds (Fig. 6), contrasted with 394.5 seconds for the controls and 1412.7 seconds for recovery of walking ability compared to 471.9 seconds for the controls.

Potentiation of pentothal sodium by prior medication with physostigmine and neostigmine resulted in increasing the anesthetic activity of a non-anesthetic dose and prolonging the anesthetic time of an anesthetic dose. These potentiations by cholinesterase inhibitors could not be blocked by 0.1 mg./Gm. of atropine, and although acetylcholine enhanced the potentiation, it alone did not produce the potentiations equal to those of neostigmine and physostigmine.

The decreases in cholinesterase (Table I) activity brought about by the inhibitors used in this study correlate quite well with the increases in activity of the CNS stimulants and depressants. DFP showed the least inhibitory activity in the dose used and the least potentiating action, while neostigmine showed the most cholinesterase depression and the greatest potentiations.

## SUMMARY

Physostigmine or neostigmine as prior medication caused potentiation in mice of the actions of strychnine and pentylenetetrazol. Subconvulsive doses were made convulsive and, in the case of strychnine, the subconvulsive dose was made lethal. In addition, the above cholinesterase inhibitors shortened the anesthetic lag time of intravenous phenobarbital sodium and increased the anesthetic activity of pentothal sodium, lengthening the anesthetic time. DFP was less effective in producing these potentiations.

Atropine sulfate (0.1 mg./Gm.) as prior medication did not block the potentiation; acetylcholine bromide (0.05 mg./Kg.) produced potentiations of much less magnitude than the reversible cholinesterase inhibitors. Thus, factors other than vasodilation must be of primary importance in these observed potentiations.

The per cent depression of cholinesterase activity by the inhibitors correlated with the degree of change in the activity of the CNS stimulants and depressants.

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# Determination of Sulfamylurea Hypoglycemic Agents and their Metabolites in Biological Fluids

By EDWARD H. WISEMAN, JOSEPHINE CHIAINI, and REX PINSON, JR.

**Amine metabolites (basic) are separated from their parent sulfamylureas and sulfamide metabolites (both acidic) by extraction. Sulfamylureas are hydrolyzed by acid to the corresponding amine for assay, under which conditions sulfamides are unaffected. Sulfamides are similarly hydrolyzed to an amine under basic conditions, sulfamylureas being stable. Flourine-containing sulfamylureas were assayed by Schöniger combustion and determination of the resultant fluoride ion.**

AS PART OF a continuing program on oral hypoglycemic agents, a series of sulfamylureas of the general formula (I) has been synthesized and studied in our laboratories (1). These studies have included attempts (2, 3) to relate the distribution, metabolism, and ex-

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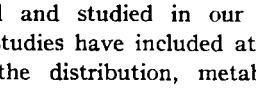

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