Estimations of the lethal dose, based on extrapolated values obtained with logarithmic-probit paper, indicated that 0.3 ml (equivalent to 0.8 mg dry weight of protein) would theoretically kill 99% of animals. All 28 mice receiving this dose died within 3 minutes. The STI solutions were given intravenously to 24, intramuscularly to 18 and subcutaneously to 10 animals. The results are given in Table I. Two endpoints were observed; (1) immediate death, and (2) a delayed reaction consisting of convulsions, or dyspnea or death within the subsequent 24-hour period. The intravenous administration of the soybean material appeared to offer the greatest degree of protection, since 88% of the group showed no reactions of either type to lethal doses of HPT. The intramuscular route offered a moderate protection providing that two hours elapsed before giving the HPT. The subcutaneous route was far less effective. When the STI and HPT solutions were incubated *in vitro* before injection, there was almost complete neutralization of the toxic effects.

In an attempt to determine the effective dosage of STI, the amount was reduced by half (*i.e.* from 18 mg to 9 mg) and given intravenously to an additional 12 mice. Two of the animals died within 5 minutes, and 9 additional mice showed delayed reactions. The lower dose level, therefore gives incomplete protection.

Conclusions. 1. When given intravenously to 4 species, the soybean trypsin inhibitor, in doses up to 0.5 g/kg, was devoid of any apparent toxic or anaphylactic effect. In the monkey, it was noted that the material caused a prolongation of the plasma coagulation time.

2. Given either intravenously or intramuscularly, the soybean trypsin inhibitor offered considerable protection against the subsequent administration of lethal doses of human placental thromboplastin, as shown in Table I.

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Inhibition of Cholinesterase by Adrenaline.

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Considerable evidence has accumulated which indicates that the neuro-humoral agents, acetylcholine and adrenaline, are not entirely antagonistic substances. Although the effector response to these two substances in most visceral structures is physiologically opposite, observations have suggested that in the central nervous system, in autonomic ganglia and at somatic motor nerve endings the one substance, adrenaline, may modify the action of the other and behave as a synergist.

Numerous experiments which have been reviewed by Burn[1] demonstrate the occurrence of this phenomenon. Dale and Gaddum,[2] in working with denervated skeletal muscle, demonstrated that the effect of acetylcholine in causing contracture could be augmented by prior administration of adrenaline. von Euler and Gaddum[3] and Bulbring and Burn[4] have similarly demonstrated increased contracture in skeletal muscle by adrenaline.

In attempting to demonstrate that acetylcholine is responsible for the transmission of impulses in the spinal cord, Bulbring and

[1] Burn, J. H., *Physiol. Rev.*, 1945, **25**, 377.

[2] Dale, H. H., and Gaddum, J. H., *J. Physiol.*, 1930, **70**, 109.

[3] v. Euler, U. S., and Gaddum, J. H., *J. Physiol.*, 1931, **73**, 54.

[4] Bulbring, E., and Burn, J. H., *J. Physiol.*, 1936, **86**, 61.

[5] Bulbring, E., and Burn, J. H., *J. Physiol.*, 1941, **100**, 337.

Burn[5] also were unable to effect motor activity by perfusing the spinal cord with defibrinated blood containing acetylcholine unless adrenaline was also added to the perfusion fluid. This experiment and others demonstrate the potentiating action of adrenaline on acetylcholine in the spinal cord. More recently Stavraky[6] reported that spinal neurones sensitized by partial isolation exhibit a period of marked excitation to adrenaline.

Of less direct evidence but highly suggestive of this same action are the general observations of increased reflex excitability in the intact animal under the influence of adrenaline and certain related sympathomimetic amines.

That adrenaline potentiates the action of acetylcholine by virtue of an inhibitory effect upon the enzyme, cholinesterase, appears likely. The experimental work reported below supports this possibility.

Experimental. Methods. The Warburg manometric technic was used employing double sidearm reaction flasks. Into the main compartment of each flask were placed definite quantities of enzyme preparation as noted below and bicarbonate-Ringer solution as prepared by Marnay and Nachmansohn.[7] One-half cc of substrate (acetylcholine, to make a 0.003 molar solution) was added to a side arm of each series of flasks. Adrenaline hydrochloride was added in varying concentrations (see below) to the other side arms. The total fluid content of each flask was 3.0 cc. After gassing with a mixture of 5% CO_2-95% N_2 and equilibrating at 37.5° C, the contents of the side arms were tipped into the reaction chamber. Controls and blanks were run simultaneously. Gas evolution resulting from the liberation of acetic acid and its action on bicarbonate was measured in the conventional manner.

Two different lyophilized preparations of enzyme of a swine source were used, the one being extracted from parotid gland and the other from caudate nucleus. These were prepared in part similar to the technic of Mendel and Mundell[8] for the purification of a pseudocholinesterase from dog pancreas. They were extracted and fractionated with $(NH_4)_2SO_4$ at 0.48 and 0.85 saturations but purification by further precipitation, adsorption and elution was not carried out. The precipitates resulting from 0.85 saturation were mixed with water, dialyzed and centrifuged. The supernatant material was then lyophilized. Nitrogen determinations by a micro-Kjeldahl technic showed that the parotid preparation contains 14.85% nitrogen. On the basis of one mg of nitrogen being equivalent to 6.25 mg of protein this preparation is about 90% protein. In a reaction flask 0.875 mg of this preparation served to liberate approximately 220 cmm of CO_2 gas in 60 minutes, its activity per unit of dry weight being raised 5-fold over that of the original tissue.

The caudate nucleus obtained from hog brain was likewise extracted, fractionated and lyophilized. Nitrogen determinations showed that it consists of 14% nitrogen indicating that this preparation is also about 90% protein. Its activity was not increased over that of fresh material and in a reaction flask 2.7 mg dry weight of preparation liberated only 130 cmm of CO_2 gas in 60 minutes. As pointed out by Mendel and Rudney[9] tissue from the central nervous system does not lend itself readily to purification.

Substrate specificity determinations give evidence that the former is a "pseudo" or "non-specific" cholinesterase since it hydrolyzes acetylcholine, benzoylcholine and several non-choline esters but does not hydrolyze acetyl-beta-methylcholine. The latter corresponds to the "specific" type of esterase. It does not hydrolyze non-choline esters, such as methyl butyrate, tripropionin, tributyrin and tricaprylin, nor does it hydrolyze benzoylcholine. It does split acetylcholine and acetyl-beta-methylcholine.

Adrenaline base was used, its solution being affected by the equivalent addition of acid to

6 Stavraky, G. W., *Am. J. Physiol.*, 1947, **150**, 37.

7 Marnay, A., and Nachmansohn, D., *J. Physiol.*, 1937, **89**, 359.

8 Mendel, B., and Mundell, D. B., *Biochem. J.*, 1943, **37**, 64.

9 Mendel, B., and Rudney, H., *Biochem. J.*, 1943, **37**, 59.

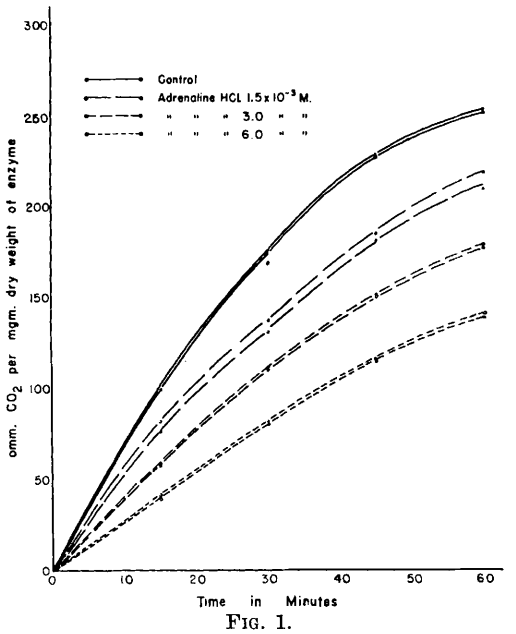

FIG. 1.

The inhibition of "non-specific" cholinesterase by varying concentrations of adrenaline HCl.

form the hydrochloride salt. Immediately after transferring it to the side arms of the Warburg flasks, the flasks and manometers were gassed to prevent its oxidation. In the experiments reported, the addition of adrenaline to the reaction chamber preceded the addition of the substrate.

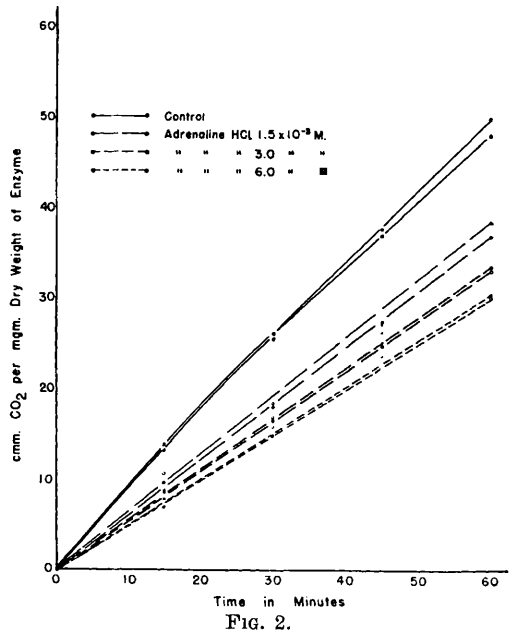

FIG. 2.

The inhibition of "specific" cholinesterase by varying concentrations of adrenaline HCl.

Results. The results of the experiment are presented in the accompanying figures. It may be seen that adrenaline inhibits the ability of cholinesterase to hydrolyze acetylcholine. The inhibition of the enzyme is immediate, commencing with the addition of adrenaline. This observation was made in instances where the addition of adrenaline to the enzyme did not precede that of the substrate. In the case of the "non-specific" esterase (Fig. 1) it may be noted that at the end of 30 minutes the inhibition is 22.2, 35.8 and 53.2% with respective 0.0015, 0.003 and 0.006 molar concentrations of adrenaline. The inhibition by 0.003 molar adrenaline approximates that produced by ephedrine in the same concentration and by eserine in a 10-7 molar concentration.

In the case of the "specific" esterase the adrenaline inhibition at the end of 30 minutes is 29.1, 35 and 40.3% with the increasing concentrations of adrenaline (see Fig. 2).

A comparison of the extent of hydrolysis at the end of 60 minutes shows that in the case of the "non-specific" enzyme the inhibition for respective increasing concentrations of adrenaline is 15.2, 29.1 and 44.4%. In the case of "specific" enzyme it is 22.9, 31.8 and 38.2%.

Only one enzyme preparation of each type has been used. The experiments with the "non-specific" enzyme have been repeated and additional determinations were made with the "specific" type of enzyme.

To eliminate the possibility of the inhibitory effect being due to the addition of the "acid" solution of adrenaline the pH of the reaction was followed in another series of flasks using a 0.003M adrenaline solution. From Table I it may be seen that at the end of 30 minutes the pH of the flask mixtures containing the added adrenaline is not less than the pH of the mixtures not containing adrenaline. At the end of 60 minutes the pH is only very slightly less in the flask containing adrenaline and substrate than in the one not containing adrenaline. It

TABLE I.
pH of Reaction Mixtures.

	30 min.	60 min.
Buffer, enzyme, substrate	7.08	6.96
Buffer, enzyme, substrate, adrenaline	7.08	6.93
Buffer, enzyme, adrenaline	7.30	7.23

should be noted that the change in pH from the 30 to the 60 minute periods is not associated directly with either the adrenaline or the substrate because it occurred in all mixtures regardless of whether or not these were present.

Discussion. The potentiating action of adrenaline on acetylcholine has been attributed to various mechanisms of action. In his review on the relation of adrenaline to acetylcholine in the nervous system, Burn[1] suggests the possibility of an anticholinesterase action of adrenaline. He also suggests, however, that the potentiating effect may be explained by altered permeability of cell membranes. Torda and Wolff[10] have demonstrated that adrenaline increases the aerobic synthesis of acetylcholine. These workers, indeed, have further suggested that both an increase in synthesis of acetylcholine and an inhibition of cholinesterase may cause certain cholinergic-like manifestations which are observable following stimulation of the sympathetic nervous system.

The anticholinesterase action of adrenaline has been reported previously, but for the most part, these reports have been of indirect evidence or confined to what is now recognized as "non-specific" enzyme. The experiments reported here elaborate upon this and in addition demonstrate a similar type of activity for the "specific" type of enzyme. This is of particular import inasmuch as it offers a mechanism for the potentiating effect of adrenaline on acetylcholine acting on the central nervous system and on preganglionic autonomic and somatic motor nerve fibers and endings.

Although the concentration of adrenaline used in this experiment is several thousand times that estimated to exist in the blood and tissues of the body the concentration of the other agents is also increased. A demonstration of parallel activity with physiological concentration would add support to the premises made.

In recognition of the finding by Torda and Wolff[10] that adrenaline increases the synthesis of acetylcholine, a twofold mechanism of action may be ascribed to adrenaline in eliciting its synergistic response. Not only is a greater quantity of acetylcholine produced, but that which is produced is protected from hydrolytic cleavage and remains to exert, within limits, a greater physiological response.

Summary. 1. Lyophilized preparations of cholinesterase, identified as "specific" and "non-specific" in type, were fractionated from swine caudate nucleus and parotid gland, respectively.

2. Adrenaline inhibits the ability of these enzymes to hydrolyze acetylcholine.

3. The potentiation of acetylcholine activity by adrenaline is correlated with the inhibitory effect of adrenaline on cholinesterase.

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10 Torda, C., and Wolff, H. G., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 86.