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## Cholinesterase Inhibition *in Vitro* by Extracts of Potato<sup>1</sup>

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While attempting to assay cholinesterase inhibitory organic phosphorus insecticide residues in various plant tissues, it became apparent that the potato (*Solanum tuberosum* L.) presented a unique problem. It was observed that aqueous extracts of potato tubers or foliage, which had not been treated with insecticide, still gave a positive test by the method employed. Hence, a more thorough study of the nature of the interference was made.

The assay procedure was based upon the measurement of inhibition of the enzyme cholinesterase by the insecticide or its metabolites (3, 5). As employed, cholinesterase catalyzed the hydrolysis of acetylcholine to acetic acid and choline, *i.e.*  $\text{CH}_3\text{COOCH}_2\text{CH}_2\text{N}(\text{CH}_3)_3\text{Cl} + \text{H}_2\text{O} = \text{CH}_3\text{COOH} + \text{HOCH}_2\text{CH}_2\text{N}(\text{CH}_3)_3\text{Cl}$ . The reaction occurred in a specially buffered medium, and the amount of acetic acid liberated in a given period of time was determined potentiometrically. The enzyme, in this case, served as a highly specific analytical reagent.

### MATERIALS AND METHODS

Plant extracts were prepared by homogenizing one part of fresh tissue with four parts of distilled water for three minutes at room temperature in a Waring Blendor fitted with hardened steel blades (Cenco Pinto blade assembly no. 17248 L54). The resulting homogenate was filtered through four layers of cheesecloth, and the volume of filtrate measured. The filtrate was brought to pH 7.35 (normal for human blood), and then diluted to 1.1 times the original volume. Extracts were usually assayed within one hour after preparation.

Outdated lots of human blood plasma were used as the source of non-specific cholinesterase (1). Various lots were pooled and stored at 5°C. Just prior to assay, a measured quantity was withdrawn, brought to pH 7.35, and diluted to 1.1 times its original volume. It was found that the cholinesterase concentration differed with various lots of pooled plasma. Hence a preliminary determination of enzyme

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concentration was made for each lot, and the quantity of plasma, usually about 5 ml, containing a standard amount of enzyme was ascertained.

The inhibition of cholinesterase was accomplished by mixing the predetermined quantities of plasma with graded aliquots, ranging from 0.5 to 10 ml, of aqueous plant extract. These were placed in volumetric flasks and diluted to 50 ml with distilled water. The contents were mixed and incubated for 70 minutes at 37.5°C (normal body temperature) in a water bath, then removed and allowed to cool to near room temperature for 15 minutes.

The assay procedure followed was essentially that of Hensel *et al.* (4) as modified by Curry (2). One ml of the inhibited enzyme was mixed with one ml of barbital buffer (pH 8.1) and 0.5 ml of acetyl choline chloride (0.264M). These solutions were placed in a 5 ml microbeaker, mixed by swirling, and the pH immediately determined with a Beckman Model G pH meter. The contents of the microbeakers were incubated for 90 minutes at 37.5°C in a water bath, then removed and allowed 15 minutes to cool to near room temperature. The pH of the contents was again determined. The difference between the initial and final pH values was proportional to the amount of uninhibited cholinesterase present, and was used with appropriate control treatments to calculate per cent inhibition.

#### RESULTS AND DISCUSSION

It was found that a plasma cholinesterase inhibitor could be readily extracted from potato foliage or tubers with water. The potency of the extract slowly decreased upon storage at 5°C, about half the activity disappearing in 10 days. The inhibitor was found to be present in potato peels at a level such that 0.5 ml of the aqueous peel extract resulted in half-maximal inhibition of the cholinesterase present in 5 ml of plasma. The inhibitor was present at approximately the same level in potato berries and tuber sprouts. The inhibitor was also detected in potato foliage and roots, and in lesser concentration in stems. In the tuber, the inhibitor was found to be concentrated in the peel, only one-tenth to one-fortieth as much existing in the innermost flesh. The inhibitor was present in nearly equal concentrations in the peelings from tubers of three varieties; Irish Cobbler, White Russet, and Red McClure.

The inhibitor is soluble in water and 95 per cent ethanol, but insoluble in ether and acetone. It does not partition into ether from water at acid, neutral, or alkaline pH values. It survives prolonged drying of whole tissues at 60° C., or brief boiling in water. It is

strongly adsorbed onto charcoal from alkaline aqueous solution, but can be eluted with 95 per cent ethanol. Hence it has properties which resemble those of quaternary ammonium bases. Certain bases, such as neostigmine and methylene blue, are known to be powerful inhibitors of cholinesterase. Preliminary experiments indicate that the substance is a much more potent inhibitor of plasma cholinesterase than of red blood cell cholinesterase. The solubility properties of the inhibitor do not resemble those of solanine or atropine, representatives of types of alkaloids which occur in certain members of the Solanaceae.

The chemical nature and physiological effects of the cholinesterase inhibitor of potato are now under study.

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