United States Patent Office 3,049,411 Patented Aug. 14, 1962

3,049,411

ENZYMATIC METHOD FOR DETECTION OF ANTICHOLINESTERASES

Charles Gelman, Chelsea, Mich., and David N. Kramer, Stevenson, Md., assignors to the United States of America as represented by the Secretary of the Army

No Drawing. Filed Feb. 11, 1959, Ser. No. 792,681
8 Claims. (Cl. 23—232)
(Granted under Title 35, U.S. Code (1952), sec. 266)

The invention herein described may be manufactured and used by or for the Government of the United States of America for governmental purposes without the payment to us of any royalty thereon.

This invention relates to a simple, highly sensitive, field detection method for the detection of anticholinesterases. It is applicable to the detection of isopropyl methylphosphonofluoridate, also known as sarin and GB, as well as other phosphorus-containing chemical warfare agents such as GA (ethyl dimethylphosphoramidocyanidate), GD (pinacolyl methylphosphonofluoridate), GF (cyclohexyl methylphosphonofluoridate), such insecticides as TEPP (tetraethyl pyrophosphate), parathion (diethyl p-nitrophenyl thiophosphate) and "Systox" (O,O diethyl 2-ethylthioethyl phosphorothioate), also, quaternary ammonium and carbamate compounds such as eserine, nesostigmine and hexamethonium.

In use, air is sampled and drawn through a wetted filter paper previously treated with cholinesterase solution. A developing reagent is then applied to the filter paper. In the presence of anticholinesterases no color develops. A deep color indicates the absence of anticholinesterases. This color will vary depending on the developing reagent used (see below). For water samples of anticholinesterases a small drop is applied to the filter paper, and then the test proceeds as outlined above.

The developing solutions contain either indoxyl acetate or an acetate of an indophenol. The detection method is based on the fact that active cholinesterase will catalyze the hydrolysis of these reagents. Anticholinesterases inactivate cholinesterases, thus inhibiting their ability to hydrolyze esters. In the case of indoxyl acetate, the active enzyme hydrolyzes it to the fluorescent indoxyl (useful for detection and analytical purposes) which is then oxidized by air to indigo. A deep blue color would indicate no anticholinesterase present. The color develops in less than 1 minute. If a ferriferro-cyanide system is present the test is accelerated. A perceptible blue-green color develops in 7 seconds, an intense color in 15–30 seconds.

A suitable developing solution is composed as follows: To 9.5 ml. phosphate buffer (pH 7.2) add 0.5 ml. indoxyl-acetate in acetone (6 mg./ml.), 4.5 mg. potassium ferricyanide and 7.2 mg. potassium ferrocyanide. The concentrations of these reagents are not critical.

A developer not including the ion salts may be prepared by adding 0.5 ml. of the acetone solution of indoxyl acetate to 9 ml. of the buffer solution.

Another developer consists of an aqueous solution of 0.1% of indophenol acetate, i.e., N-(4′ acetoxyphenyl)-p-quinone imine, plus the phosphate buffer. In this case the enzyme hydrolyzes the somewhat yellow acetate to the deep purple indophenol.

Other colorless acetates of indophenols (hydrolyzable by cholinesterase to highly colored compounds) can be used in this detection method. Examples of such acetates of indophenols are: 2,6 dichlorindophenyl acetate; 2,6 dibromo indophenyl acetate; N-(8 acetoxy-5′-quinolinyl) p-quinone imine and its 2,6 dichloro- and dibromo-derivatives; N-(4′ acetoxy-3′-methyl) 2,6 dibromo-p-quinone imine.

Enzyme papers are prepared by first impregnating filter paper with the phosphate buffer (pH 7.2), drying, then a 4% solution of cholinesterase in phosphate buffer (pH 7.2) in bovine albumin, gelatin or other proteins. The concentration of enzyme is adjusted to fit the sensitivity desired. Results cited here were obtained with papers having 25 activity units (Winthrop and Stearns) bovine red cell cholinesterase on a ¼″ disk. The type of filter paper is not critical and choice would vary according to application.

The phosphate buffer referred to above is formed by mixing 0.1 N NaOH and 0.1 M K_2HPO_4 aqueous solutions to give the desired pH. The detection method is successful over the pH range 6.4 to 8.5. It is preferable to employ approximately pH 7.2 to 7.5.

The enzyme paper may also be prepared by the method of Fleischer et al., Analytical Chemistry, vol. 27, pp. 1080 to 1083 (1955). The hemoglobin employed by those authors should, however, be omitted to avoid color interference.

Other porous supports, such as organic and glass fibrous mats and silica gel may be substituted for the filter paper.

Two types of detector unit have been made and tested. One type consisted of a ¼″ disk of methylene blue filter paper mounted in a plastic disk. Another consisted of a disk of alpha web paper inserted in a 6 mm. glass tube. The enzyme treated papers were wetted with water and then air was drawn through them. They were immediately treated with one or more drops of the developing solution and exposed to the air. As little as 0.002 mmg. (microgram) GB in air could be detected, completely inhibiting the enzyme and preventing development of the color.

Enzyme papers should be stored in a dry atmosphere. The activity will be retained for extended periods of time in this manner even at 65° C.

The test is not dependent on a change in pH as other proposed enzyme-detection schemes have been. The test is not subject therefore to interferences by acidic products present in the atmosphere. The test is fast and simple. The test is not affected by bleach vapors nor by chemical warfare agents which are not of the anticholinesterase type, such as mustard gas.

We claim:

1. A method for the detection of a compound of high anticholinesterase activity which comprises contacting a fluid containing said compound with a porous support impregnated with cholinesterase and buffered to a pH in the range of 6.4 to 8.5 then developing said support with a developer consisting essentially of an aqueous solution buffered to a pH in the range of about 6.4 to 8.5 and comprising a compound selected from the group consisting of acetates of indophenols and indoxyl acetate.

2. A method as defined in claim 1 wherein said developer consists essentially of an aqueous solution of indoxyl acetate and a buffer salt.

3. A method as defined in claim 1 wherein said developer consists essentially of an aqueous solution of indophenyl acetate and a buffer salt.

4. A method for the detection of a compound of high anticholinesterase activity which comprises contacting a fluid containing said compound with a porous support impregnated with cholinesterase and buffered to a pH of about 7.2, then developing said support with a developer consisting essentially of an aqueous solution buffered to a pH to the range of about 6.4 to 8.5 and comprising an acetate of an indophenol.

5. A method as defined in claim 4 wherein said developer consists essentially of an aqueous solution of indophenyl acetate and a buffer salt.

6. A method for the detection of a compound of high anticholinesterase activity which comprises contacting a

fluid containing said compound with a porous support impregnated with cholinesterase buffered to a pH of about 7.2 and then developing said support in a developer consisting essentially of an aqueous solution buffered to a pH of about 7.2 and comprising indoxyl acetate.

7. A method as defined in claim 6 wherein said developer consists essentially of an aqueous solution of indoxyl acetate and a buffer salt.

8. A method as defined in claim 6 wherein said developer consists essentially of an aqueous solution of indoxyl acetate, potassium ferricyanide, potassium ferrocyanide and a buffer salt.

References Cited in the file of this patent

UNITED STATES PATENTS

2,865,719 Kramer ---------- Dec. 23, 1958