Using forceps, the muscles in the neck region are separated in the mid-line until the trachea is exposed. The internal carotid artery is now isolated and a ligature passed under it. The animal is now held head downward and, using the ligature as a guide, the artery is cut and the blood permitted to flow into the tube as shown in the arrangement in Fig. 2. We have found that the tube illustrated permits obtaining more serum than is ordinarily possible in the average centrifuge tube.

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## THE SILVER IODIDE TEST FOR HYDRO-CYANIC ACID

It is not infrequently desirable to ascertain the presence of hydrocyanic acid in faint traces, with the use of a delicate, fairly rapid and simple test. The ready recognition of this compound is of use occasionally to agriculturists, toxicologists, health officials, fumigators of ships and buildings, and to the coroner, as well as to numerous scientific investigators engaged in various types of work.

Recently the writer has had occasion to make tests for traces of HCN in connection with current studies of cyanogenetic glucosidases present in certain marine organisms. With the cooperation of Dr. C. E. ZoBell of this institution, a number of marine bacteria and fungi are being studied with reference to the capacity of these forms to hydrolyze the cyanogenetic glucoside amygdalin. Similar studies are being made of the alimentary tracts of certain marine invertebrates.

Guignard's test, which consists of the suspension of sodium picrate papers over the solution or culture under investigation, is very delicate, but according to the numerous writers, not highly specific for HCN. This test, however, is employed as a preliminary one in our laboratories to indicate the presence of free HCN in various microorganism cultures or enzyme systems. If the compound is present, the bright yellow color of the alkaline picrate paper is converted, through various stages of yellow-orange, orange and red orange to the brick color of the reduced compound, sodium picramate.

In order to confirm the presence of HCN, and to demonstrate that it, and not other substances, is responsible for the reddening of the papers, an additional delicate and specific test has been developed, based, in principle, upon the quantitative method for determining HCN described and used by Roe<sup>1</sup> and later by Bishop.<sup>2</sup> This method, used in determining the amount of HCN present in certain leaves, kernels, and pure cyanogenetic plant glucosides, consists in aerating the solution containing the digest, and conveying the air thence into dilute potassium hydroxide to catch all of the HCN, aeration being continued until all of the compound has been carried over. The alkaline cyanide solution is finally titrated for CN<sup>-</sup>, using the general method of Liebig, with very dilute silver nitrate solution, employing potassium iodide as an indicator. With proper lighting and a black background the end point can be determined very sharply as the first permanent faint cloud of AgI appears, indicating that all of the CN<sup>-</sup> has been taken up in forming the complex salt potassium argenticyanide KAg(CN)<sub>2</sub>.

The procedure used in our own work, for confirming the presence of HCN indicated by Guignard's test, consists in a modification, perhaps better termed an inversion, of Roe's method.

To a thoroughly cleaned test-tube are added first one drop of 5 per cent. KI solution, and one drop of .001 M AgNO<sub>3</sub> (solutions as dilute as .00025 M can be used if only the faintest traces of HCN are suspected), and then one cubic centimeter of 5 per cent. KOH solution. A faint bluish cloud of AgI is now present. Air is drawn through the system or culture which is being tested for HCN, and thence into the alkaline silver iodide suspension. If HCN is present, the KCN formed dissolves the AgI, rendering the solution perfectly clear. The test is specific for HCN, since H<sub>2</sub>S, NH<sub>3</sub>, HCNS (NaCNS), and other bacterial catabolites were found to exert no visible effects upon the silver iodide, which is soluble only in such non-volatile compounds as Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, NaCl, etc. It is important that the reagent be prepared freshly for each test, since AgI does not remain in the colloidally dispersed condition described under the circumstances for longer than a day at most. The limit of delicacy is about 1 part HCN in 2,000,000, when .00025 M AgNO<sub>3</sub> is used under ordinary laboratory conditions. Without doubt the perception of even fainter clouds of AgI with the use of special nephelometric instruments would render the test still more delicate.

An attractive feature of this method, besides its specificity, delicacy and simplicity, is that, should a positive test for HCN be found, quantitative determination of the compound can be made forthwith if desired by merely continuing the aeration without altering the system or interrupting the process, thus making use of the chemical method perfected by Roe.

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<sup>2</sup>L. R. Bishop, "The Estimation of Cyanogenetic Glucosides," Biochem. Jour., 21: 1162, 1927.

<sup>&</sup>lt;sup>1</sup>J. H. Roe, "The Estimation of the Hydrogen Cyanide Content of Amygdalin by the Aeration Method," Jour. Biol. Chem., 58: 667, 1924.