DETECTION OF GELATIN IN AGAR.[1,2]

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The U. S. P. X test for the detection of gelatin in agar reads as follows:

"A solution made by boiling 0.1 Gm. of Agar in 100 cc. of water and cooling yields no precipitate with tannic acid T.S."

This test has been reported to us as yielding a positive reaction with agar known to contain no gelatin. Agar belongs to the galactan group of carbohydrates and should not react with the protein reagents. In this investigation the effects of the common protein reagents on agar and agar adulterated with gelatin were studied.

EXPERIMENTAL.

Reagents.—The behavior of the following reagents upon solutions of agar and agar containing gelatin was determined: Copper sulphate T.S.; mercuric nitrate T.S.; tannic acid T.S., and also a saturated aqueous solution; picric acid T.S., a saturated aqueous solution and a saturated alcoholic solution; bromine T.S.; mercuric potassium iodide T.S.; phosphotungstic acid (1:10) and trichloracetic acid (1:4). The following special reagents were also used:

Millon's Reagent.—Dissolve 1 part, by weight, of mercury in 2 parts, by weight, of nitric acid and dilute with 2 volumes of distilled water.

Robert's Reagent.—Add 1 volume of nitric acid to 5 volumes of a saturated aqueous solution of magnesium sulphate.

Tanret's Reagent.—Dissolve 1.35 Gm. mercuric chloride in 25 cc. of water, add 3.32 Gm. potassium iodide dissolved in 25 cc. of water, dilute to 60 cc. with water and add 20 cc. of glacial acetic acid.

Samples.—Three samples of agar were available, all of which were asserted to be free from gelatin. The nitrogen content of the samples, determined by the Kjeldahl method, were:

Sample.	% *N*.	% Protein (*N* × 6.38).
A	0.20	1.276
B	0.13	0.829
C	0.0355	0.226

These were used in the proportion of 1 Gm. dissolved in 100 cc. of boiling distilled water, and are designated as Solutions A, B and C.

Solution D.—1 Gm. of gelatin dissolved in 100 cc. of boiling water.

Solution E.—1 Gm. of Sample A containing 10% of gelatin dissolved in 100 cc. of boiling water.

Solution F.—1 Gm. of A containing 5% of gelatin dissolved in 100 cc. of boiling water.

Solution G.—1 Gm. of A containing 1% of gelatin dissolved in 100 cc. of boiling water.

Solution H.—1 Gm. of A containing 0.1% of gelatin dissolved in 100 cc. of boiling water.

Solution I.—1 Gm. of A containing 0.01% of gelatin dissolved in 100 cc. of boiling water.

Solution J.—1 Gm. of B containing 1% of gelatin dissolved in 100 cc. of boiling water.

Solution K.—1 Gm. of B containing 0.1% of gelatin dissolved in 100 cc. of boiling water.

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[1] Published by permission of the chairman of the Committee of Revision, U. S. P. XI.

[2] Scientific Section, A. PH. A., Madison meeting, 1933.

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All of the tests were performed at a temperature of 40–50° C. unless otherwise specified.

The tests and results may be described as follows:

Tannic Acid T.S. added drop by drop produced a pronounced turbidity in Solutions A, B and C, and a precipitate in the remaining solutions. The sensitivity was not especially changed by the use of a saturated solution of tannic acid.

Millon's Reagent added drop by drop produced a turbidity in Solutions A, B and C, and a precipitate in the remaining solutions, which became slightly pink on heating.

Mercuric Potassium Iodide T.S. and *Bromine T.S.* added drop by drop produced neither precipitate nor turbidity.

Phosphotungstic Acid added drop by drop produced a slight turbidity in Solutions A, B and C, and a white precipitate in the remaining solutions.

Trichloroacetic Acid produced no precipitate with the pure agars, nor when the gelatin concentration was 0.1% or less. In the presence of larger amounts of gelatin a white precipitate was produced.

Tanret's Reagent yielded no precipitate with the pure agars. A faint turbidity was produced by 0.01% of gelatin, while the higher concentrations formed a precipitate.

Robert's Reagent produced a precipitate only when the concentration of gelatin was 1% or greater.

Biuret Reagent.—To 5 cc. of the agar solution was added an equal volume of potassium hydroxide (1:4) and, after mixing, 3 drops of copper sulphate T.S. The solutions of pure agar showed only the color of the reagent. When 1% or more of gelatin was present the characteristic purple color of copper biuret was produced. With the lower concentrations of gelatin the blue color of the copper reagent was modified by a slightly purple tint.

Picric Acid T.S. was added dropwise to the agar solution and the mixture allowed to stand. Saturated aqueous and alcoholic solutions of picric acid were also added dropwise to separate portions of solution. A distinct yellow precipitate was formed in every instance when gelatin was present, while Solutions A, B and C remained clear.

CONCLUSION.

Tannic acid produced a precipitate with pure agar and as a result it cannot be used as a test for gelatin. Picric acid will precipitate gelatin but will not precipitate agar and is a very good reagent for the detection of gelatin. An alcoholic solution of picric acid is more sensitive toward the gelatin than an aqueous solution.

Tanret's reagent is not as sensitive toward gelatin as picric acid. It will precipitate gelatin but not agar. The biuret test is obtained with gelatin and samples of agar containing over one per cent of gelatin.

Millon's reagent is a common protein reagent, but all proteins do not react with the reagent. It will form a white precipitate with agar containing gelatin.

No precipitates could be obtained with the metallic salts. The best results were obtained with picric acid solutions and Tanret's reagent.

In the authors' opinion, the U. S. P. should prescribe the following test for gelatin in agar:

Dissolve 1 Gm. of Agar in 100 cc. of boiling distilled water and allow to cool to about 50° C. To 5 cc. of the solution add 5 cc. of picric acid T.S.; no turbidity appears within ten minutes (*gelatin*).

PITTSBURGH, PA.

November 9, 1933.