tives have been prepared. Of chief interest among these is 5-*n*-butyl-5-ethyl-barbituric acid which is a powerful hypnotic. Curiously enough, the substitution of a phenoxyl group for either of the alkyls in this substance destroys its physiological activity.

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DETECTION OF THYMINE IN THE PRESENCE OF SUGAR

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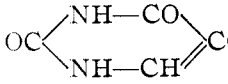
Received April 26, 1922

In a recent paper in THIS JOURNAL, Johnson and Baudisch[1] have described a new test for the detection of the pyrimidine, thymine,



which is specific and much more sensitive than any method hitherto suggested. Johnson and Baudisch showed that on treatment with ferrous sulfate and sodium hydrogen carbonate in the presence of air, thymine is oxidized to urea, pyruvic acid, acetol and possibly formic acid. Uracil and cytosine also give urea when treated in a like manner, but only thymine is capable of giving acetol and pyruvic acid by virtue of the methyl group in the 5 position of the pyrimidine ring. In the absence of sugar, the formation of pyruvic acid, or acetol, and urea by this oxidation is sufficient to prove the presence of thymine. Inasmuch as one of these oxidation products, namely acetol, also arises in the distillation of the simpler carbohydrates with sodium hydrogen carbonate,[2] the acetol test cannot be applied for thymine in the presence of sugar. Since sugar is present in the nucleic acid molecule, it is important to have a reaction to detect small quantities of thymine in the presence of sugar.

For the detection of urea, acetol and pyruvic acid after the oxidation of thymine, the precipitated ferric hydroxide is filtered off and the filtrate distilled. The distillate, which contains the acetol, has a sweet smell and reduces an ammoniacal solution of silver nitrate. Acetol can be identified in small quantities by the use of the test first discovered by Baudisch[3] and discussed in another paper.[2] This procedure involves heating the distillate with *o*-amino-benzaldehyde in alkaline solution with the consequent production, by condensation with acetol, of 3-oxyquinaldine. This compound is readily identified by its characteristic blue fluorescence in sodium hydrogen carbonate solution.

[1] Johnson and Baudisch, THIS JOURNAL, **43**, 2670 (1921). Baudisch and Johnson, *Ber.*, **55**, 18 (1921).

[2] Baudisch and Deuel, THIS JOURNAL, **44**, 1585 (1922).

[3] Baudisch, *Biochem. Z.*, **89**, 279 (1918).

On distillation of the acetol, as described above, pyruvic acid remains as the sodium salt in the residue along with urea. Pyruvic acid may readily be identified by heating some of the residue with *o*-nitrobenzaldehyde in potassium hydroxide solution. Indigo blue, which is identified most readily by extraction with chloroform, is formed in this reaction. Urea can be determined quantitatively by precipitation with xanthydrol, a method which has been utilized for the determination of the amount of breakdown of the thymine molecule by this and similar oxidizing agents.

The procedure which was found to be satisfactory for the detection of thymine in the presence of sugar involves the separation of thymine from the solution tested by precipitation as the mercury salt. The detailed method is given below. Blank determinations carried out with a 0.1% glucose solution showed that no glucose was carried down with the mercury precipitate.

Sensitiveness.—The determination of the sensitiveness of these tests was studied and it was found that a distinct fluorescent reaction was obtained with 5 mg. of thymine when it was oxidized without preliminary precipitation with mercuric chloride. Besides a distinct test for acetol, a faint pyruvic acid test was obtained on the residue. The urea test was much more sensitive than either of these other tests, quantities of thymine as small as 0.5 mg. being readily detected when the thymine was oxidized directly without preliminary precipitation.

When a preliminary precipitation is required with mercuric chloride as is the case when sugar is present, 10 to 15 mg. of thymine in 100–150 cc. of solution is required to give a fluorescence which can be seen in daylight. Under similar conditions 30 mg. of thymine on oxidation gave a strong pyruvic acid test, and the limit of sensitivity of this test probably lies between 10 and 15 mg.

The fluorescence of 3-oxyquinaldine is much more readily seen in light of short wave length such as that given by the iron arc light. This affords a method of seeing the fluorescence at night, since it is not visible in ordinary artificial light. By the use of the iron arc light, as small quantities as 1 mg. of thymine can be detected either by oxidation of the thymine directly or following preliminary precipitation.

Experimental Part

The description of a single experiment will suffice to give a more exact conception of the method of carrying out this test. To 100 cc. of a 0.1% sugar solution, 0.03 g. of thymine was added and dissolved. The thymine was precipitated as the mercury salt on the addition of 10 cc. of saturated mercuric chloride solution and sufficient sodium hydroxide solution to make the mixture distinctly alkaline. The precipitate was separated by centrifuging for about 10 minutes and then after pouring off the super-

natant liquid the precipitate was thoroughly mixed with about 100 cc. of distilled water and centrifuged again. Blank experiments carried out in a similar manner with a sugar solution in the absence of thymine showed that no sugar was precipitated by the thymine. That the sugar remained is solution was confirmed also by the fact that the supernatant liquid after the first centrifugation gave a strong positive acetol test for sugar on distillation with sodium hydrogen carbonate. The small quantity of liquid mechanically held with the precipitate after the second centrifugation did not contain sufficient sugar to give a positive acetol test, since as large a quantity as 10 cc. of the supernatant liquid after the second centrifugation failed to give a positive test for acetol. After pouring off the supernatant liquid as thoroughly as possible following this second centrifugation, the precipitate was suspended in about 100 cc. of water and decomposed with hydrogen sulfide. The mercuric sulfide was then filtered off and the excess of hydrogen sulfide removed by boiling. The liquid remaining was cooled and made up to 100 cc.

The oxidation of the thymine was brought about according to the method first used by Baudisch[4] by shaking the final solution with 10 g. of sodium hydrogen carbonate and 10 g. of finely pulverized ferrous sulfate. This was carried out by shaking the mixture in a liter flask connected with an air condenser. Ferrous carbonate was first formed as a white precipitate but soon turned gray, then green and finally changed to the characteristic brownish-red color given by ferric hydroxide. The oxidation was then completed, the time required in this experiment being 45 minutes. After filtering off the ferric hydroxide, the filtrate was distilled. The distillate, which contains the acetol, was heated in a beaker over a free flame after the addition of enough sodium hydroxide solution to make it distinctly alkaline, and then of 0.003 g. of *o*-amino-benzaldehyde dissolved in a little alcohol. A piece of porous plate was added to prevent bumping. After evaporation to about $^1/_3$ volume, the solution was cooled and enough hydrochloric acid added to make the solution distinctly acid. At this point, the solution was shaken with ether once or twice to remove substances which caused cloudiness and so might hide the fluorescence. Then the solution was made alkaline by adding solid sodium hydrogen carbonate and with the amount of thymine used in this experiment a marked fluorescence was obtained. This fluorescence test was confirmed by extracting the fluorescent 3-oxyquinaldine with alcohol-free ether, drying the ether solution with some sodium sulfate, removal of the ether by distillation and dissolving the residue of 3-oxyquinaldine in warm alcohol. On the addition of water to the alcohol solution the fluorescence appeared with considerable intensity.

The residue remaining in the distilling flask after distillation of the

[4] Baudisch, *Ber.*, **54**, 406 (1921).

acetol amounted to about 20 cc. and was used for the detection of pyruvic acid and urea. It usually contains a considerable amount of iron oxides which are easily removed by centrifuging for a few minutes. If a large amount of thymine was originally present so that sufficient urea and pyruvic acid were formed to allow detection of these substances in $^1/_2$ of the supernatant liquid, one part (A) can be used for the detection of pyruvic acid and the other part (B) for the detection of urea.

For the detection of pyruvic acid, a small amount of *o*-nitrobenzaldehyde, preferably in the form of an emulsion, and a few cubic centimeters of sodium hydroxide solution were added to the liquid to be tested. After thorough agitation the mixture was warmed to facilitate the formation of indigo blue. After cooling and shaking with chloroform the indigo blue is readily seen in the layer of this solvent.

For the detection of urea, Part B should be diluted considerably so that, on the addition of glacial acetic acid, sodium acetate will not be precipitated. After dilution of the clear liquid to about 75 cc., two volumes of glacial acetic acid are added. To this was added an excess of a 5% alcohol solution of xanthydrol. With large quantities of urea, a precipitate of dixanthyl urea is formed immediately but small quantities of urea, especially in dil. solutions, may not precipitate for several hours. Dixanthyl urea occurs as a typical white precipitate which melts at 250–260°.

Application.—Practical application of this test for thymine has been made in the examination of the pyrimidines obtained from the nucleic acid of the *tubercle bacillus*, which has just been completed by Prof. Johnson and co-workers in the Sheffield Laboratory of Organic Chemistry. A strongly positive acetol test was obtained as well as a test for urea after oxidation of 0.0044 g. of the thymine fraction. The amount of dixanthyl urea obtained after oxidation indicated a 52% breakdown of the thymine molecule. The melting point of the dixanthyl urea was found to be approximately 250°.

Application of this test for the detection of thymine in the urine is being studied.

Summary

A modification of the method, described recently by Johnson and Baudisch, for the detection of thymine, is given which makes it applicable in the presence of sugar. Ten to 15 mg. of thymine may readily be detected in the daylight, while as small quantities as 1 mg. may be detected by the use of the iron arc light.

The authors wish to acknowledge the helpful suggestions and criticisms of Professors Lafayette B. Mendel and Treat B. Johnson during the progress of this work.

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