IMPROVED PHENYLHYDRAZINE TEST FOR FORMALDEHYDE.

BY A. B. LYONS.

In an exhaustive study of the tests which have been proposed for the detection of formaldehyde, Gettler* assigns high rank among color tests to those in which phenylhydrazine is employed as a reagent. The most distinctive of these is that in which sodium nitroprusside is used conjointly with the phenylhydrazine. These reagents are added in succession to the highly dilute formaldehyde solution without noticeable immediate effect. The further addition of sodium hydroxide in slight excess produces in the solution at once a blue color which soon gives place to a succession of hues ending in a comparatively permanent red.

The reagents used may conveniently be made of the following concentrations: Phenylhydrazine hydrochloride, 1%; sodium nitroprusside, 2%; sodium hydroxide, 5%. Of the first two, the quantity required is a single drop—as measured by a pipette which delivers to each cc. 32 drops of distilled water at 25° C. A convenient quantity of the suspected aqueous solution is 0.5 cc. or less of a solution containing 0.04% of formaldehyde to which may be added 1 cc. of distilled water. In case turbidity is produced within one minute on adding the reagents, a smaller quantity of the sample should be used for the test. At all events, it is best in any case in which incipient precipitation is observed before the sodium hydroxide is added, to make a new test, diminishing the quantity taken of the sample.

The color changes through greenish to amber shades, becoming finally red. The time required for completing the cycle of changes varies from one or two to twenty minutes or so. I believe that it may be conceded that this is the most satisfactory color test that we possess, becoming practically conclusive if confirmed by the egg albumen and apomorphine tests.

A second phenylhydrazine test, devised like the foregoing by A. Muth, employs with the phenylhydrazine, ferric chloride together with hydrochloric acid. Details of the routine for carrying out this form of the test are not at hand. Empirically I have arrived at the following as a practical *modus operandi*. To 1 cc. of the suspected sample add from a pipette which delivers to the cc. 32 drops of water, 1 drop of a 1 per cent. aqueous solution of phenylhydrazine hydrochloride (freshly prepared and filtered), 1 drop of a five per cent. solution of ferric chloride $(FeCl)_3$, 3 drops of a ten per cent. solution of sodium chloride and 5 drops (from a pipette which delivers 70 drops to 1 cc.) of sulphuric acid. More or less rapidly the solution develops a vivid red color, the tint depending on the quantity of formaldehyde present.

For reasons not evident the depth of color varies in an erratic manner even when care is taken to adhere strictly to the routine procedure selected. A fairly strong reaction may be expected in a solution containing one part in 25,000 of formaldehyde, and this is by no means the limit. In some experiments I have obtained a recognizable color when the solution was of only one-fourth that strength.

The ferric chloride in this test may be replaced by almost any soluble ferric salt, including the soluble phosphate, the solution containing the same iron percentage.

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* *Journal Biological Chemistry*, 42, 311-328, 1920.

The red color of the solution is not discharged by mere dilution with water, as in the peptone or egg albumen tests. It is, however, nearly or quite destroyed by supersaturation of the acid by sodium hydroxide, a precipitate of ferric hydroxide being thrown down.

The test may be advantageously modified by substituting for the ferric chloride, potassium ferricyanide—one drop of a 1% solution to 1 cc. of the sample tested, or a suitable dilution thereof. The characteristic red color appears promptly and generally in greater intensity than when ferric chloride is used.

The behavior of the red compound towards neutral solvents is interesting and gives us confirmatory evidence of its identity. If the red solution is shaken out with petroleum ether, its color is at least in part discharged. The shaking out may be repeated with several successive portions of petroleum ether, the color of the solution thus obtained being orange-yellow. Excess of acid may be washed out of this yellow solution by repeated shaking out with distilled water. Otherwise the solution may be rendered neutral or even alkaline and then extracted by shaking out with petroleum ether. The ethereal solution in either case is to be evaporated in a porcelain capsule. If the solution is free from acid, the residue will be of a yellow or orange color. If the acid has not been all washed out the residue when completely dry will show more or less of a violet-red color. If the residue is wholly yellow or orange colored, bring close to the interior of the capsule the stopper of a bottle containing strong nitric acid, and watch the change to violet-red of portions of the yellow residue.

This constitutes so far as I can learn a wholly new feature in the phenylhydrazine test for formaldehyde and suggests the possibility of increasing indefinitely the sensitiveness of the test, by simply operating on a relatively large quantity of the suspected sample. We may use, instead of 1 cc. of the solution, 10 or 20 cc. concentrating thus correspondingly the color indication of the presence of formaldehyde —possibly even enabling us to separate thus a definite chemical compound known to result from the reaction of phenylhydrazine with formaldehyde.

Still another modification of the phenylhydrazine test I have worked out which resembles in its results that just described. In this ammonium persulphate is employed in place of potassium ferricyanide. Sulphuric acid is used as in the other forms of the phenylhydrazine test but there is distinct advantage in using also a small quantity of a chloride furnishing thus potentially hydrochloric acid.

In case of a solution containing 1: 2500 of formaldehyde, use 1 cc. of the solution, 3 drops of a 10% solution of sodium chloride, 2 drops of a 2% solution of ammonium persulphate and 10 drops of strong sulphuric acid, these proportions subject to modification in the light of further experiments. The color resulting is not distinguishable from that produced in the foregoing form of the test. The red solution may be extracted with petroleum ether in the manner above described, observing, however, that more water may be required to remove the acid on account of the rather sparing solubility of the ammonium persulphate.

Some other oxidizing agents may be substituted for those I have experimented with—possibly to advantage.

It remains to study the practical adaptation of the tests under discussion to the detection of methyl alcohol in alcoholic beverages.

SUMMARY.

Among the most useful color tests for detection of formaldehyde are those in which phenylhydrazine is a principal reagent.

This paper describes two new modifications of one of these color tests. In the first potassium ferricyanide takes the place of ferric chloride, having the advantage of rapidity of operation as well as fuller development of the characteristic color.

In the second the subsidiary reagents are ammonium persulphate and sodium chloride. In both sulphuric acid instead of hydrochloric acid is used.

An original method of isolating the red coloring substance by extraction with petroleum ether or similar immiscible solvent is described, applicable to all three forms of the phenylhydrazine test No. 1 of A. Muth.

LABORATORY OF NELSON, BAKER AND CO.,
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THE USE OF INSULIN IN THE TREATMENT OF DIABETES MELLITUS.*

BY ROBERT W. KEETON.

It is proposed in this paper to discuss very briefly, from a historical viewpoint, the methods available for the treatment of diabetes prior to the introduction of insulin, the mechanism of insulin action, the clinical situations in which insulin has proven valuable, and a few questions of practical interest to the pharmacist.

DIABETIC TREATMENT PRIOR TO INSULIN THERAPY DIET RESTRICTION—FASTING.

This period extends from the time of the earliest investigators to 1919 and 1920. It was characterized by a wide variety of methods of management. The very multiplicity of these methods suggested that the key to the problem was still hidden. Out of these attempts grew some very thoroughly established clinical facts upon which all these methods were based. These may be stated:

1—Carbohydrates added to the diet of a diabetic who is showing sugar in the urine cause an increase in urinary sugar.

2—Fats added to the diet may cause the appearance of acetone in the urine or increase the quantity if it is present.

3—The diabetic gains in tolerance, if his urine can be maintained sugar-free. Conversely when sugar is persistently present in the urine the tolerance of a patient decreases.

4—Uncontrolled diabetes is a progressive disease and in the young often advances to a rapidly fatal termination.

With these facts before them the students of the subject were led, in all of their efforts to combat the disease, to the principle of diet restriction. The quantity of food fed must fall within the limits of the patient's tolerance. Van Noorden [1] was accustomed to use one or two fast days to prevent coma and he describes the immediate rapid fall of acidosis resulting. He spoke of these days quite aptly

*Read before November meeting of the Chicago Branch of the American Pharmaceutical Association.