CXXXII. AN IMPROVED AND SIMPLIFIED BENZIDINE TEST FOR BLOOD IN URINE AND OTHER CLINICAL MATERIAL.

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THE principle of the benzidine test for blood, as is well known, depends upon the peroxidase action of haematin, the benzidine being oxidised to a blue compound. The great drawbacks to the test are the instability of the hydrogen peroxide solution and its impurities. Ozonic ether is often substituted for hydrogen peroxide on account of its greater stability but of course it is much more expensive. Theoretically hydrogen peroxide is the perfect oxidising agent, but the instability of aqueous solutions, together with the impurities—acids, sulphates, phosphates, and alkali metals—which are generally present, detract considerably from its usefulness in practical laboratory work, and blank tests are always necessary to control results.

About 24 years ago Tanatar [1908] produced a solid compound of hydrogen peroxide and urea which was stable, neutral and ashless, and free from inorganic impurities, and under the registered name of "Hyperol" it is available on the market at a low price. It contains 35 % of hydrogen peroxide, remarkably pure—the only impurity being the merest trace of citric acid which, however, is insufficient to affect titrations with methyl orange—and perfectly stable. It only begins to decompose when heated to 60°, and the decomposition results in the formation of oxygen, water and urea.

The stability, purity and strength of hyperol render it a most useful, reliable and powerful oxidising agent. As a powder it is easy and convenient to handle and will keep indefinitely provided it is not brought into contact with moisture. Containing 35 % of hydrogen peroxide, it is much more powerful than the 10 volume (3 %) aqueous solution which is generally used in laboratories.

In the benzidine test for blood hyperol can be used, instead of the hydrogen peroxide solution or ozonic ether, with considerable saving of expense, rendering the test much more delicate and reliable, and so simplifying the technique that in most cases it can be done as a "spot" test.

Method as applied to urine.

1. Place a small amount of benzidine (enough to cover the tip of a pocket-knife blade) on a clean white plate.
2. Add two drops of glacial acetic acid.
3. Add one drop of urine.
4. Add a small amount of hyperol (enough to cover the tip of a pocket-knife blade).

A bright deep blue colour appears immediately if blood be present, even in a very minute trace.

Sources of error. Urine containing potassium iodide, pus or enzymes will react to this test in the same way as with the ordinary guaiacum or benzidine tests. If pus or enzymes be suspected the urine should first be heated to over 60° (boiling is not necessary) and cooled before the test is applied. No reaction with pus or enzymes will be obtained under these circumstances.

If potassium iodide be suspected add a drop of dilute sulphuric acid (10–25 %) to the blue mixture when the blue colour will disappear. Next add a drop of fresh 1 % starch solution and the blue colour will reappear if due to iodides but will fail to reappear if due to blood.

Another and simpler method of detecting iodides is to place a drop of the urine on the white plate, without any benzidine, and add a small quantity of hyperol and then a drop of 1 % starch solution. A blue colour will result.

Marked alkalinity or acidity has practically no effect on the hyperol test whereas marked alkalinity prevents the development of the reaction for blood in the guaiacum test.

Relative sensitivity of guaiacum, ordinary benzidine and hyperol-benzidine tests for blood in urine.

From a large series of tests the following results were obtained:

	Guaiacum	Ordinary benzidine	Hyperol-benzidine
Smallest quantity of blood detectable	From 1 part in 1000 to 1 in 10,000	From 1 part in 25,000 to 1 in 60,000	From 1 part in 200,000 to 1 in 260,000

Thus with the hyperol-benzidine test described one can be sure of detecting 1 part of blood in 200,000 parts of urine as compared with the 1 part in 1000 revealed with the guaiacum test. It is of course much more sensitive than the spectroscope.

The test may be applied to stomach contents, cerebro-spinal and other body fluids under the same conditions as with urine.

Blood in faeces. Rub up a small quantity of the faeces—about the size of a pea—with 2–3 cc. of water in a test-tube, and heat to over 60°, then add 2–4 drops of glacial acetic acid, shake and cool. Take one drop of the watery extract and test as with urine.

In testing faeces for blood it is much better, however, to make and test an ethereal extract as follows.

Rub up a small quantity of faeces with 2–3 cc. of water in a test-tube and heat on a water-bath to over 60° for about 5 minutes, then cool and add 2–4 drops of glacial acetic acid. Shake the mixture and then add about 3 cc. of ether and shake again. Test the separated ethereal extract in the same way as urine.

Much greater sensitivity can be obtained by decanting the ethereal extract and evaporating it on a water-bath at 60°, then treating the residue with one or two drops of distilled water and testing this moistened residue. This latter method can of course be applied to body fluids. In the case of urine it has been found that, by centrifuging the specimen and examining the deposit in this fashion, 1 part of blood can be detected in 3,000,000 parts of urine. Iodides, pus or enzymes do not interfere with the test when performed in this way.

Summary.

1. "Hyperol," a dry stable powder, containing 35 % of hydrogen peroxide, can be used as the oxidising agent in the benzidine test for blood in clinical laboratory practice.

2. By its use the performance of the test is simplicity itself and the cost is almost negligible.

3. The test so performed is thoroughly reliable and extremely sensitive.

REFERENCE.

Tanatar (1908). *J. Phys. Chem. Soc. Russ.* **40**, 376.