A NEW REAGENT FOR DETECTING OCCULT BLOOD

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BOAS, in 1901, was the first to call attention to the importance of the chemical reactions for occult blood and their great help in the diagnosis of obscure conditions. Before that time, one relied on the microscope or the spectroscope, or on the making of hæmin crystals, all of which processes are infinitely less delicate and more difficult to carry out than the chemical tests. Nowadays the chemical testing for occult blood is a part of the routine examination in any case where stone in the kidney, duodenal ulcer, or malignant disease of the intestinal tract, is suspected. Benzidin, up to the present, has been easily the most satisfactory reagent for clinical work, but in ortho-tolidin we have a new reagent that is even more satisfactory on account of its greater delicacy, its more lasting colour, and the fact that it can be made into a solution that retains its delicacy unimpaired for three or four weeks. All this so simplifies the carrying out of the test that it might well take a place beside the familiar tests for albumin and sugar in the equipment of the busy practitioner.

The substance is Tolidin or orthotolidin—a crystalline basic body of the aromatic series with melting point 129-130°C., very slightly soluble in water, easily soluble in alcohol and æther and closely allied to toluidin and benzidin. The properties and derivatives of this base were first described by one of us in 1886.* It has never been used as a reagent for the detection of occult blood, for which purpose it is admirably adapted. It has been found to be an exceedingly delicate means of detecting the presence of blood in a watery solution, and has several important advantages over the other usual reagents when used in clinical work for the detection of blood in the excretions and secretions of the body.

To obtain some idea of its value as a clinical reagent it was tested against guaiacum, benzidin, and phenolphthalin, first of all with watery solutions of blood and then with blood in urine, fæces, and stomach contents. Its advantages in clinical use were then recognized, as it seemed to be less hindered in its action by the inhibitory substances in the body fluids than the other reagents.

*"Proceedings of British Association, 1886"—R. F. Ruttan.

I might explain briefly these chemical reactions for occult blood. They depend on the so-called peroxidase activity of the blood, and to perform the test, some substance (leuco-body), such as guaiacum, benzidin, phenolphthalin and tolidin is used that becomes coloured when oxidized, and an oxidizing agent is also used (as ozonized æther, hydrogen peroxide, etc.). These substances are placed together in a test-tube (in solution); no change takes place; a little blood is added, when the characteristic colour of the oxidized substance employed appears immediately. This is due to the ability of the blood, by virtue of its so-called peroxidase activity, to seize upon the oxygen of the oxidizing agent, and to carry it over and deliver it to the chromogenic substance—so bringing about a characteristic colour reaction, i.e., a positive reaction. Many other substances, besides blood, may give this reaction; but, when properly performed and controlled, it can only be brought about by blood—or rather by the iron-containing blood pigment.

When examining the secretions and excretions of the body, we have to take many precautions. Many substances, such as pus, mucus, and various animal and vegetable ferments, which can give a positive reaction, have to be excluded. This is done by boiling; and, further, in the case of fæces, the hæmoglobin of a meat diet, which of course can give a positive reaction, has to be guarded against by dieting the patient with milk and vegetables for from thirty-six to sixty-five hours, depending on the presence or absence of diarrhœa. Or it is better still to "mark off" the stools by giving carmine. Another source of difficulty is that the secretions and excretions may contain substances that have a reducing action, that is, they are capable of seizing upon and appropriating the oxygen of the oxydizing substance, so that none is available to bring about the oxidizing of the reagent, and so a positive reaction.

In the carrying out of the tests, the reagents were made up in the following way: guaiacum was used in strength of 1-25 in methylated spirits; benzidin and tolidin in solutions of similar strength, but in glacial acetic acid. Pure phenolphthalin was prepared in the way recommended by Kastle (in *Bul.* 51 *of the Hyg. Dept. U.S.A.*). Considerable difficulty was experienced in making up this low reagent; extreme cleanliness had to be observed and the reagent had to be kept in a cool, dark place. Even then it lasted only some forty-eight hours in good condition.

A blood solution of known strength was used, made by taking one gm. of crystalline hæmoglobin and dissolving it in a litre of

distilled water, making a solution of 1-1000, and from this solution all the other higher dilutions were made. Hydrogen peroxide was used as the oxidizing agent in all the tests, a chemically pure Merck preparation, Perhydrol, being diluted down to about a three per cent. solution—the strength of the ordinary commercial product. In testing, 1 c.c. of the reagent was used, and 1 c.c. of the substance to be tested, and, in all cases, 1 c.c. of hydrogen peroxide, except when the phenolphthalin solution was used which already contained it.

The relative delicacy of the different reagents for blood in a watery solution was first tested. It was found that guaiacum detected blood in dilutions of 1 to 50,000; benzidin, of 1 to 700,000; tolidin, of 1 to 7,000,000; phenolphthalin, of 1 to 10,000,000 and even more.

As an agent for detecting blood in watery solution phenolphthalin was found to be much the most delicate, in fact the test had to be carefully controlled to be sure the positive reaction was due to blood, for it was found that the ordinary distilled water of the laboratory gave a pinkish colour—positive reaction—due to some infinitestimal trace of iron or copper salt, and so all the distilled water had to be redistilled in glass before being used. Guaiacum and benzidin, when positive, give a prompt reaction which, however, depending on the dilution, does not last very long. With tolidin, the colour, a green to a blue black, depending on dilution, does not develop quite so rapidly, appearing more gradually and increasing in intensity, the colours lasting a much longer time, even several hours.

The delicacy of the different reagents in detecting blood in urine, fæces, and stomach contents was then compared.

Blood in Urine. The 1 to 1,000 watery solution of blood, which was used before, was diluted with an equal quantity of normal (negative) urine, making a blood solution of 1 to 2,000. For the higher dilutions normal urine was added to this 1 to 2,000 solution. In this way it was found that blood in urine was detected by guaiacum and benzidin in dilutions of 1 to 6,000, the reaction of benzidin being very slightly the more marked, but still only slight and lasting only a very few minutes, while tolidin detected it in a dilution of 1 to 24,000, giving with this dilution a deep greenish-blue colour that lasted half an hour or more. Phenolphthalin, however, failed to give a positive reaction when a solution of blood in urine of 1 to 2,000 was added.

Fæces. For the detection of blood in fæces, the stool of a

patient who had been on a meat-free diet for ten days, was used. This stool was dried and a two per cent. emulsion of it made, boiled, and one c.c. added to the different reagents, after which one c.c. of blood in watery solution was added to this mixture. The emulsion gave alone a negative reaction with all reagents, while the stool of a healthy person on full meat diet gave a strongly positive reaction even after being boiled. Testing in this way it was found that guaiacum could detect blood in dilutions of 1 to 10,000; benzidin and tolidin, of 1 to 100,000; while phenolphthalin again proved to be a very poor clinical reagent, detecting only dilutions of 1 to 2,000, and this only when additional hydrogen peroxide was added to the Kastle's reagent.

Stomach Contents. When testing for blood in stomach contents, the material withdrawn from the stomach after an ordinary test meal was used. This had a total acidity of 74, free HCl 45, and contained no lactic acid. One c.c. of this material was added to each reagent before the blood in watery solution was added. Here phenolphthalin proved to be of no value, as it is necessary for it to be in an alkaline solution and even with additional alkali it was less delicate than guaiacum. Guaiacum just detected blood in dilutions of 1 to 5,000; benzidin and tolidin in dilutions of 1 to 30,000.

To sum up, we have in orthotolidin a reagent of very great delicacy for detecting blood in watery solution, and, in addition to this, it is superior to the reagents in general use for clinical work. It is greatly superior to all the reagents for detecting blood in urine, and while benzidin, which is easily the best of the well-known reagents for clinical work, is quoted here as being equal to tolidin for the detection of blood in fæces and stomach contents, this is true only for freshly prepared solutions of benzidin, older solutions losing fifty per cent. of their delicacy in twenty-four hours, whereas tolidin will remain unchanged for from three to four weeks, an important fact, adding to the ease with which the test can be carried out. Another point in favour of tolidin is that when the blood is in small quantity, the reaction increases gradually in intensity and persists longer than with the other reagents, so being more easily read. There are, of course, many ways of increasing the delicacy of the reactions for occult blood when testing urine and fæces—such as treating with acetic acid and filtering or extracting with alcohol, æther, etc., but all these things tend to make the reaction longer and more complicated, and, where alcohol and æther are used, more expensive, so that it is a great advantage to get a reliable, delicate reagent that we can apply directly to the fluid to be tested.