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SOME INVESTIGATIONS ON THE DETECTION OF OCCULT BLOOD IN STOOLS,

with a Simplified Technique for the application of the Benzidin Test.

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A great deal of work has been done in the past in the investigation of stools for Occult Blood, that is to say for the presence of quantities of blood either too small in amount or too much changed in its passage through the alimentary canal to be recognisable by ordinary macroscopic or microscopic means. A great deal of the earliest investigations aimed at the elaboration of a test which should be delicate enough to detect a small trace of blood, and yet not too delicate to be inconvenient in practice. At the present day the only test for Occult Blood in general use is the Benzidin reaction which has been shown by Clark[1] in his experiments with every one of the familiar methods of investigating for Occult Blood, i.e. the Guaiacum, Spectroscopic etc., to be incomparably superior to any others.

So completely has this circumstance been demonstrated by Clark and other observers, notably Cammidge[2], that no useful purpose would be served by any experiments

[1] "The Detection of Blood Pigment in the Faeces" - St Bartholomew's Hospital Reports, 1909.

[2] "The Faeces in Children and Adults."

which simply traverse the same ground; and the primary objects of the investigations embodied in this Thesis were

1. To experiment with the technique as originally laid down by Schlesinger and Holst.
2. To vary the conditions of the patient's diet, particularly in accordance with such restrictions that the tests should not be invalidated.
3. To determine the minimum quantity of blood which ingested into the alimentary tract would yet give a positive reaction in the faeces.

The technique of the Benzidin reaction now in use ("Schlesinger and Holst's modification") is as follows:- A "knife point" of Benzidin (Merck) is put into one test tube with 2 c.c. of Glacial Acetic Acid (B.P.). This should form a supersaturated solution. To this is now added"a few drops" of a faecal emulsion which has been previously boiled. Hydrogen Peroxide (10 vols.) i.e. B.P. is now added, 2½ c.c. to 3 c.c. being employed. A positive reaction is a deep green or blue coloration within one minute. This modification obviates a preliminary and irksome treatment of the stool. The delicacy of the test is somewhat impaired by this modification,

but this for clinical purposes is not a disadvantage.

It has been found by other observers that, simple though this test is, it must be performed with a close approximation to quantitative accuracy because, through a curious anomaly, a large quantity of the faecal emulsion may give a negative reaction, whereas a smaller amount may give a positive. On the other hand, excess of Hydrogen Peroxide may, through solution of the blue colouring matter, give a negative reaction in a positive case.

My first endeavour, then, was to place this test on a strict quantitative basis, and with this end in view, and following the lines suggested to me by Captain Abrahams, R.A.M.C., Medical Specialist, Connaught Hospital, Aldershot, I manipulated all the reagents, including the faecal emulsion (which it is obvious is inevitably of indefinite strength) with carefully graduated pipettes. A second advantage of working by this method was the very great chemical cleanliness which is possible on all occasions, a factor of great importance in a test in which a contamination with previous material, so slight as to be measured by hundreds of thousandths, absolutely vitiates any result obtained.

The third advantage I claim by this method is greater delicacy. I have frequently performed this test in the manner I describe below, and contrasted its results on the same faecal emulsions or dilute solution of blood, with the test as performed by Schlesinger and Holst's method. The fourth advantage is that of economy of reagents. During the present crisis Benzidin (a German preparation) has been not only scarce but almost unprocurable. In my modification the amount of Benzidin used is reduced to a minimum, and is only a fraction of that required in the ordinary test tube method, in which a "knife point" of Benzidin is used.

My technique is as follows:-

1. Apparatus required:

(a) A saturated solution of Benzidin in Alcohol.

(b) Glacial Acetic Acid, B.P.

(c) Hydrogen Peroxide, B.P. (10 vols).

(d) Several glass measuring pipettes, (easily made from glass tubing) fitted with rubber teats.

(e) A small porcelain slab, with for preference small round shallow wells.

A preliminary investigation proceeded on the lines of standardising the delicacy of the reagents employed.

So delicate is the reaction that, frequently, using two drops of H_2O_2 will destroy the blue colour given by one drop of H_2O_2.

A measured quantity of blood (20 c.mm.) was diluted to form a 1 in 1000 solution with distilled water. From this as a stock solution, solutions 1 in 5000, 1 in 10,000 and so on up to 1 in 100,000 were prepared.

It will be manifest that, by working with these blood solutions of known strength, an exact standard was obtainable on the following lines: If a positive reaction occurred when the following were placed in one of the wells of the slab, viz:-

(a) One drop of the Benzidin solution

(b) One drop of Glacial Acetic Acid

(c) One drop of Blood Solution of 1 in 50,000

(d) One drop of H_2O_2

then the delicacy of the test is 1 in 200,000.

Without dealing at length with the details of this part of the work, I may state finally that I was able to detect beyond any question, a blood dilution of 1 in 240,000. A dilution of 1 in 10,000 yielded an intense positive reaction, as intense as almost pure blood. By

varying the quantities of the reagents employed, I found that an optimum result was produced by using -

2 drops of Benzidin solution

2 drops of Glacial Acetic Acid

1 drop of the solution being tested

1 drop of H_2O_2

The delicacy obtained by using these proportions (1 in 240,000), although it compares quite favourably with that obtained by other observers using Schlesinger and Holst's method, is, I venture to think, more delicate than that method, as well as more accurate. It is of course considerably cruder than the Phenolphthalein Test of Boas, which is said to give a positive reaction with a dilution of 1 in 8,000,000, but the latter test is quite unsuitable for clinical purposes, not merely on account of the complexity of the technique but from the minuteness of its specificity.

As is of course well known, a patient whose stools are to be tested must be kept on a diet which excludes all exogenous blood, and is at the same time carefully investigated for the presence of any source of endogenous haemorrhage which might reach the alimentary canal. The first requirement is met by restricting the patient

to a haemoglobin-free diet. In practice this is held to exclude meat in every form, animal soups, fish, chicken etc. At the same time the diet must be free from substances such as green vegetables and from certain drugs, (notably Potassium Permanganate) which in themselves are capable of producing a positive reaction. It is generally stated that three days on a haemoglobin-free diet are sufficient. In my opinion a conclusive result is not possible unless a longer interval is allowed, preferably 5 days, to elapse to allow of all haemoglobin-containing substances being certainly eliminated from the body. It is most convenient to place patients whose stools are to be tested on a Milk Diet, which includes milk, bread, milk puddings, arrowroot, oatmeal etc.

It is interesting to observe, en passant, that even milk itself occasionally gives a faint positive reaction, though I am not satisfied that this was not due to contamination by blood, probably from the cow's teats, possibly from the milkers' hands, inasmuch as a negative reaction was produced in the majority of samples of milk tested. The oxydase which occurs in milk is, like other oxydases, destroyed by boiling. As regards fish,

a faint positive reaction was given by emulsion of fish meat in water. In every case, however, in which I tested the stools of patients on a Milk Diet including fish (cod, plaice and hake, of which 12 ounces is the allowance in Army Hospitals daily) a negative reaction was obtained. From which I conclude that the exclusion of fish is an unnecessary refinement, although it is perhaps as well always to err on the side of undue carefulness.

It was interesting and instructive to find, when testing a number of so-called "meat extracts" of commerce that while some gave, as expected, an intense positive reaction, others yielded a negative result, thus demonstrating in the latter an entire freedom from any animal tissues. Properly made soups and meat extracts contain plenty of haemoglobin, and since the patient on a Milk Diet may acquire the impression that the taking of soup does not matter (an opinion which his nurses may confidently support), it is urgently advisable not only to lay down the most exact instructions to prohibit absolutely the patient partaking of anything outside the prescribed diet, but also to enquire for reassurances that soups, meat extracts, beef tea

essences etc., have not been taken. It is generally held that Iron Salts yield a positive reaction, but in my experience this is not the case. Bismuth does not yield a positive reaction, and this is naturally of some importance, as patients likely to undergo such an investigation are frequently on Bismuth compounds. A very large number of drugs in common use were also tested from time to time, as patients undergoing medical treatment were utilised for the observations now to be recorded, i.e. those with acute rheumatism and other conditions which rendered the prolonged administration of a Milk Diet no hardship. As these patients were undergoing treatment with various drugs, it was necessary of course to exclude any possibility of contamination from such a source.

The final purpose of my investigations, and comprising the greatest part of the work done, was to ascertain the amount of blood which ingested into the alimentary canal would give a positive reaction in the stools. I have already stated that my reagents, as used, were sensitive to a dilution of 1 part of blood in 240,000, but it is manifestly impossible to say how much blood in a person's stomach will be required to appear in a stool

still capable of giving a positive reaction, bearing in mind the extraordinary opportunities for dilution which occur in the voluminous alimentary tract. So far as I am aware no previous attempt has been made to determine this amount, and I have read a statement that the smallest scratch or abrasion of the walls of the alimentary tract is sufficient to produce enough blood in the stools to give an intense positive reaction, a statement which my results clearly show to be a gross exaggeration.

During my investigations a number of difficulties not anticipated soon presented themselves. First, it is often impossible to retain any one patient for an indefinite period on a Milk Diet, though the exigencies of such investigations may demand regular daily tests over a period of several weeks, since sufficient time must be allowed between the different amounts of blood given for the stools again to become negative on at least two successive occasions. Again, the loss of any one particular stool may similarly waste a certain number of days until the conditions can be repeated. Contamination, as by the administration of some prohibited article of food, is a far more fruitful source of trouble and delay, even in hospital, than would be

supposed.

In such an investigation it was imperative that every stool from the patient under observation should be passed into an absolutely clean vessel, and that every motion should be examined, so that the series should be complete. In a military, as in civil hospitals, orderlies and nurses are only on duty during certain hours, and much of my earlier work was stultified by overworked or incompetent orderlies neglecting to follow the orders to save all stools of the patients under investigation, the throwing away of a single motion in many cases rendering the whole series of tests incomplete. Another difficulty discovered was that much depended on the consistence of a motion, soft or semisolid excreta giving the most constant results. Some of the patients treated only had a motion at intervals of two days or more, and in such cases it was found that the blood administered might be confined to one scybalous mass, corresponding to the meal after or before which the blood had been administered. Another difficulty in such cases was that sometimes the individual scybala were coated with a mucus "varnish" containing blood, and so giving a positive reaction for blood. In

all such cases care was taken to remove the test specimen from the inside of a scybala.

On every occasion when these tests were performed (which was daily over a long period) controls were always performed to ensure that the reagents used were not inert, as might possibly be suggested on any day on which all the stools tested happened to be negative.

My criterion of a positive result was not the production of an intense Prussian-blue colour, but a faint blue or definite (if faint) green colour was considered as a positive.

In all cases the patient's own blood was administered, to imitate the condition of haemorrhage from a gastric or duodenal ulcer.

I found that a positive reaction was not obtained until V drops of blood (m$\overline{\text{V}}$) in a single dose was ingested. Although certain patients gave a positive reaction with m$\overline{\text{ii}}$, I do not feel justified in giving less than m$\overline{\text{V}}$ as a minimum quantity which yields a positive reaction in any case.

The administration of one drop (1 minim) of blood three times daily also yields a positive result, so that it is evident that comparatively small repeated

haemorrhages, as from a gastric ulcer, ought to be readily detected with proper precautions, although such a figure is a very long way from the previously referred to statement that the amount of blood from a mere scratch of the mucus membrane of any part of the alimentary tract will cause a positive result. I would again add that the reaction aimed at was very faint. It is true that in all cases an unmistakable reaction was obtained (controls being always performed before any result was accepted as positive), but in the majority of my cases in which a dose of 5 minims of blood was given an unmistakable blue reaction was obtained.

I am perfectly satisfied that from 2 to 5 minims of blood will produce a clearly positive reaction in the majority of people. Certain anomalous cases in which 5 minims or even 10 minims of blood failed to produce a reaction were very occasionally observed. Such cases would be exceedingly difficult to explain if one were obliged to suppose a perfectly homogeneous admixture of the administered blood to occur in the alimentary canal, but since the anomalies referred to occurred in cases who were chronically constipated, it

is more than likely that the dose of blood administered became inspissated and confined to one portion of stool which escaped examination. It was impossible under working conditions to take samples from every scybala and only specimens from different portions could be examined. Such a case is quoted in detail, No. 2.

The exact details of blood administrations and results obtained in the two representative cases given below will explain better than in any other way the manner in which I have arrived at the foregoing conclusions:-

Case 1 - J.S., aet 20; suffering from Acute Rheumatism, and having been on a Milk Diet 7 days before the tests began.

Date	Amount of blood given	Time	Time of Motion	Result of Test
18/2/16	. . .	. .	1st 12.35 p.m. 2nd 2.15 p.m.	Neg. Neg.
,,	5 minims	1.30 p.m.	. . . .	. .
20/2/16	. . .	. .	Thrown away	. .
21/2/16	. . .	. .	3.45 p.m.	Neg.
22/2/16	5 minims	4.30 p.m.	. . . .	. .
23/2/16	. . .	. .	1.20 p.m.	Pos.
25/2/16	. . .	. .	11.30 a.m.	Neg.

Date	Amount of Blood given	Time	Time of motion	Result of Test
25/2/16	2½ minims	1.30 p.m.	. . . .	. .
26/2/16	. . .	. .	2 p.m.	Pos.
28/2/16	. . .	. .	9 a.m.	Neg.
,,	2 minims	1.15 p.m.	. . . .	. .
29/2/16	. . .	. .	1st 7.15 a.m.	Pos.
,,			2nd 10 a.m.	Pos.
1/3/16	. . .	. .	9 a.m.	Neg.
,,	1 minim t.i.d.	1.30 p.m.	. . . .	. .
2/3/16	. . .	. .	2 p.m.	Neg.
3/3/16	. . .	. .	1st 8.45 a.m.	Pos.
,,	. . .	. .	2nd 7 p.m.	Pos.
4/3/16	. . .	. .	1st 5 a.m.	Pos.
,,	. . .	. .	2nd 4.35 p.m.	Pos.
5/3/16	Stopped m.1 t.i.d.	9 a.m.	. . . .	. .
,,	. . .	. .	4.25 a.m.	Pos.
6/3/16	. . .	. .	6.30 a.m.	Neg.
8/3/16	. . .	. .	5.30 a.m.	Neg.
,,	1 minim	6.30 p.m.	. . . .	. .
9/3/16	. . .	. .	6 a.m { Scybala / Pultaceous	Neg. / Trace Pos.
,,	. . .	. .	5.30 p.m.	Neg.
10/3/16	. . .	. .	5.25 a.m.	Neg.

Case 2 - W.B., suffering from Dyspepsia, Milk Diet 5 days previously.

Date	Amount of Blood given	Time	Daily Motions	Results of Tests
20/11/15	. .	. .	8.30 a.m. .	Neg.
,,	minims XX	1.30 p.m.	. . .	. .
,,	. .	. .	6.30 p.m. .	Neg.
21/11/15	. .	. .	5 a.m. .	Pos.
,,	. .	. .	6.30 a.m. .	Pos.
,,	. .	. .	11 a.m. .	Pos.
,,	. .	. .	12.30 a.m. .	Pos.
,,	. .	. .	6.20 p.m. .	Pos.
22/11/15	. .	. .	9 a.m. .	Pos.
23/11/15	. .	. .	4.30 a.m. .	Neg.
,,	. .	. .	9 p.m. .	Neg.
24/11/15	. .	. .	12.15 p.m. .	Neg.
,,	minims X	1.30 p.m.	. . .	. .
25/11/15	. .	. .	11.30 a.m. .	Neg.
,,	. .	. .	1.30 p.m. .	Neg.
27/11/15	. .	. .	1.30 p.m. .	Neg.
,,	. .	. .	12 p.m. .	Neg.
28/11/15	minims XV	1.30 p.m.	. . .	. .
,,	. .	. .	1.15 p.m. .	Neg.
,,	. .	. .	3.30 p.m. .	Neg.

Date	Amount of Blood given	Time	Daily Motions	Results of Tests
29/11/15	. .	. .	1.15 p.m.	Pos.
30/11/15	. .	. .	9 a.m.	Neg.
,,	minims X	1.30 p.m.	. . .	. .
1/12/15	. .	. .	8.30 a.m.	Pos.
,,	. .	. .	8.30 p.m.	Neg.
2/12/15	. .	. .	7.15 p.m.	Pos.
3/12/15	. .	. .	12.30 p.m.	Neg.
,,	minims V	2 p.m.	. . .	. .
4/12/15	. .	. .	3.20 p.m.	Neg.
,,	. .	. .	5.10 p.m. Very	Pos.
5/12/15	. .	. .	1.15 p.m. loose	Pos.
6/12/15	. .	. .	4.45 p.m. motions	Pos.
7/12/15	. .	. .	8.10 p.m.	Pos.
,,	. .	. .	7 p.m.	Neg.
8/12/15	. .	. .	11.56 a.m.	Neg.
,,	minims V	1.30 p.m.	. . .	. .
9/12/15	. .	. .	4.15 a.m.	Pos.
,,	. .	. .	3.15 p.m.	Pos.
10/12/15	Patient discharged from Hospital.			

Summary:

In the investigations, as recorded above, I have endeavoured

1. To simplify, on a strictly quantitative basis, the application of the Benzidin Test so as to make it one easily within the power of even a busy general practitioner, or where the use of a microscope or spectroscope would be inconvenient. Such a test has even a wider use than the detection of occult blood in faeces, and I have used it in the daily ward work of the Connaught Hospital for the rapid detection of blood pigment where such was suspected, e.g.

(a) In the urine from a case of suspected Paroxysmal Haemoglobinuria.

(b) In several cases where coloured cerebrospinal fluid was obtained on lumbar puncture

(c) In samples of alkaline urine, in which the presence of blood was suspected. (If such urine is to be microscopically examined for blood a fresh specimen must be used. On keeping for any time, any red blood corpuscles present are soon disintegrated.)

2. To ascertain the minimum quantity of blood which, if present in the alimentary canal as the result of one haemorrhage (as in a gastric or duodenal ulcer) or from a number of small repeated haemorrhages, would be sufficient to produce a positive reaction in the stools of the patient. My investigations lead me to place the former, in the majority of persons, as five minims; and the latter as one minim. In all such cases, of course, the contamination of the stools from other sources must be excluded.