Original Articles.

COMPARATIVE TESTS FOR OCCULT BLOOD IN GASTRIC CONTENTS AND FECES, WITH ESPECIAL REFERENCE TO THE BENZEDIN TEST.

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THE demand in recent years for a sure and convenient clinical test for minute quantities of blood or "occult blood"* (Boas¹) has led not only to discovery of new tests but to the modification and improvement of the older methods. Formerly, aside from the Teichmann test which was of little clinical value, a macroscopical examination was the only means adopted to determine the presence of blood in the stools, and it is only within the past four or five years in this country that other methods have been more generally employed. Any one who has used more exact tests will have found that in many stools very considerable quantities of blood may be present and be imperceptible to the naked eye. Schumm² states that stools with a blood content of 5% and more are often passed by as blood-free, and that on a plentiful milk diet, free of meat, even a larger percentage may be unnoticed. The same is true in the examination of gastric contents when, in the presence of free hydrochloric acid, there may be no visible evidence of blood, and yet a distinct reaction for blood be obtained. When the stomach contents are first withdrawn the presence of moderate quantities of fresh blood may be plainly seen, but the hemoglobin, in the presence of the acid gastric juice, is soon broken up and a solution of hematin is formed, causing the pink or red color to fade or disappear; or where large quantities of blood are present, to assume a brown color. Both Kuttner³ and Ewald⁴ call attention to this fact, and the occurrence is illustrated by the following case of subacute alcoholic gastritis: A patient brought to the clinic a specimen of red fluid with a grumous sediment which he had vomited two days previous, — the blood tests were intensely positive. Five hours after ingestion of a glass of milk, a stomach tube with closed end was easily swallowed and 15 ccm. of mucoid opalescent contents obtained; no food elements were seen, and there was not the slightest visible evidence of blood, yet the guaiac, aloin and paraphenyldiamin tests were all clearly positive. Free HCl was present.

It is not the purpose of this paper to consider the clinical value of detecting occult bleeding already proved by many observers (as Kuttner³ Boas,¹ Boas and Kochman,⁵ Schloss,⁶ Clemm,⁷ Hartmann,⁸ Rossel,⁹ Schmilinsky,¹⁰ Petrachi¹¹ and Rüttemeyer,¹² and in this country, Steele,^{13 15 16} Steele and Butt,¹⁴ Tileston,¹⁷ White,¹⁸ and Friedenwald and Rosenthal,¹⁹) but to show by the follow-

ing review of statistics and a series of comparative tests the methods best suited for clinical purposes.

TEICHMANN'S TEST.

It is more than fifty years since Teichmann,²⁰ working under Henle, after a long series of experiments, obtained hemin crystals, and demonstrated the process of their formation. Similar experiments on the blood had been independently carried on by Kunde²¹ and Hunde,²² and previously reported. Since that time Teichmann's hemin test has been, until recently, the chief method for detecting small quantities of blood. Hemin crystals, according to Hagar,²³ are produced in a blood dilution of 1 to 20,000; Thierfelder's analysis makes one part hemin correspond to about 162 parts of blood. It is not a sure test, nor convenient, and it is less delicate than other tests. Schumm² (p. 10) says that until 1902 he had frequently employed Teichmann's test, but could not recommend it for the detection of blood in stools as it sometimes completely failed, even when quite large quantities of blood were present, and in other cases he obtained a positive result only after many repetitions of the test. There seem to be fewer objections to its use as a test for blood in gastric contents.

SPECTROSCOPIC BLOOD TEST.

This method has long been employed to detect blood in solutions. But, as stated by Boas and Schmilinsky, the apparatus is too expensive and cumbersome for use by the general practitioner, and less delicate than the guaiac test. The cyanide of potassium test of Hofmann²⁴ and the hemocromogen test as employed by Hoppe-Seyler,²⁵ are probably the most delicate tests by means of the spectroscope. Jaworski and Korolwicz,²⁶ Schlesinger and Holst,²⁷ Schumm,² and Grünwald²⁸ also review the spectroscopic methods. Grünwald particularly advocates the cyanide of potassium test and gives the following table showing the relative delicacy of tests:

	In a Dilution of	
Weber's (his own results)	1: 2,000 (blood solution)	positive
Benzedin	1: 150,000	" " "
Hemocromogen	1: 5,000	" " "
Cyankalium	1: 5,000	" " "

But, as shown below, we know that the guaiac test is more delicate than indicated in this table.

Schmilinsky (p. 2145†) gives a simple method of obtaining the blood spectrum by means of the hematoporphyrin method: a number of drops of stomach contents or feces, well mixed with water, are slowly added to a few cubic centimeters of concentrated sulphuric acid. If the solution is placed in a glass dish with plain parallel walls the spectrum can be easily recognized. The test is quite sharp and quickly carried out; if it is negative the guaiac test, which is more delicate, must be applied, so nothing is gained by its use.

* Termed by Boas "occult bleeding" because blood is neither macroscopically nor microscopically demonstrable, but can be detected by chemical means.

† The principle, of course, is that of Hoppe-Seyler, and the sides of the vessel (known as the hematinometer) are 1 cm. apart.

GUAIAIC TEST.

The guaiac test, next to Teichmann's, is the oldest clinical test devised for the detection of occult blood. Schönbein²⁹ discovered the test and established a majority of the facts relating to it; but at the same time His³⁰ determined many facts regarding the test, and a few years later van Deen³¹ as the result of his experiments (published in 1863) proved the delicacy of guaiac as a test for blood, determined many of its limitations, and pointed out its value as a test in forensic medicine. Liman,³² also, in this year (1863) reviews the work of van Deen and partially that of Schönbein. He made more than 50 applications of the guaiac test to various blood dilutions and other substances. In the past twenty-five years the results of all these experimenters have been many times tested and reaffirmed by various writers.

Schönbein showed that the iron of the blood was the means of transferring the ozone of the ozone-carrier (as H_2O_2) to the tincture of guaiac. His (p. 213) remarked that the active substance of blood corpuscles, when dried or when a solution is heated to boiling, does not lose the quality of causing the blue color to appear; he heated a solution of blood corpuscles to boiling, then caused complete coagulation by adding a drop of acetic acid, and this coagulum, which was then washed with distilled water, still gave a blue color with guaiac and H_2O_2 , and even the colorless filtrate gave a weak color reaction. His found that there was no difference between the activity of venous and arterial blood in causing a blue color on addition of tincture of guaiac and H_2O_2 ; but he found that no color appeared if the ozone bearer (Ozonträger) was first added to the blood solution and then the tincture of guaiac; but if to this mixture H_2O_2 was again added, a blue color quickly appeared. Schönbein (p. 213) found that pure blood serum caused no color change with guaiac, and washed fibrin only a slight reaction; bile and saliva gave an uncertain cloudy blue; chyle, fresh milk, casein (action destroyed on boiling), milk, sugar, epidermis, gastric mucosa, pancreas and liver substance, all gave a doubtful color; egg albumin,¹ long macerated raw flesh, and fresh urine no reaction; fat and connective tissue, uric acid and urea a weak color.

Schönbein and van Deen both applied the guaiac test to a large number of metallic substances and the following are the chief preparations mentioned by van Deen: the oxide and sub-carbonate of iron gave negative results; the ferrous salts and sulphide of iron gave a slight degree of color reaction; the acetates and citrates and chlorides, particularly the latter, gave this reaction in more marked degree; the subacetate and sulphate of copper gave slight reactions similar to that of old blood; some of the reactions though not typical were sufficient to confuse; other copper salts, the red oxide of lead, coloring matters, as carmine and logwood, also sandal wood, all gave negative results.

In one of the many experiments with blood and guaiac van Deen found that when a portion of a three-year-old dried blood-cake (of calves) was mixed in water to a dilution of at least 1 to 40,000, the guaiac and old oil of turpentine still gave a positive reaction, though it was necessary to wait some moments for the blue color to appear; he found the test was equally sensitive for old or fresh blood. Liman³² (p. 205) obtained no perceptible guaiac reaction in a blood dilution of 1 to 40,000.

It is surprising that, after so much experimentation with guaiac as a blood test, little mention was made of it till Vitali³³ published his paper in 1887. Then followed articles by Brücke³⁴ (1888) and Schuster³⁵ (1890). Vitali³³ found the test reacted to pus as well as blood. On account of the many exceptions to its use as a blood-test it had little clinical value till modified by Weber³⁶ in 1893. Weber found that by making an acetic acid ethereal extract, practically all the substances which may interfere with the test for blood are eliminated, particularly the preparations of iron, pus, saliva, milk and starch, or their action much weakened as in the cases of greens, spices and bile. The presence of bile in stools may obscure the trace of blue or green by brown shades when the amount of blood is small, and especially if bile is in excess. Schumm's method (see below) overcomes this objection. Brandenburg³⁷ found that when guaiac was added to pus it gave a blue color reaction without the addition of an ozone-bearing substance. Brandenburg found this reaction with pus was due to the nucleo-albumin which was precipitated by acetic acid, and that an acetic acid ethereal extract of pure pus gave no color reaction with guaiac; so that pus does not interfere with the blood test by Weber's method.

Besides the writers mentioned, many others have contributed to the knowledge of this test, including Kuttner,³ Boas,³⁸ Boas and Kochman,⁵ Schaer,³⁹ Hartmann,⁴⁰ Joachim,⁴¹ Carlson,⁴² Ewald,⁴ and Mathieu and Roux,⁴³ and in this country within the past four years, Steele,¹³ and Steele and Butt,¹⁴ Benedict,⁴⁴ * Tileston,¹⁷ White¹⁸ and Cowie.⁴⁵

Although Kuttner³ had, in a measure, indicated the value of detecting small amounts of blood in stomach contents, Boas was the first to make general applications of the test for occult blood in affections of the gastro-intestinal tract.

The following are Weber's original directions: (1) A plentiful portion (about 5 gm.) of feces is rubbed up with water, (2) a portion of this mixture is treated with one-third volume of glacial acetic acid and (3) extracted with ether. (4) After clearing, a few cubic centimeters of the ether extract are poured off and to this are added about 10 drops

* Benedict's modification of the guaiac test consists in adding to about 2 to 5 gm. of feces (diarrheal consistency) an equal amount of gasoline, then add about 1 ccm. fresh tincture of guaiac and an equal amount of old oil of turpentine. If blood be present, the gasoline, (which rises above the emulsion of feces, guaiac and turpentine) is colored green or blue according to the amount of hemoglobin present. Benedict claims for the test avoidance of delay and the spurring incident to the ether acetic acid extraction. The ingredients may be added in any order. The same objections, however, obtain with this test as with the original van Deen test.

of tincture of guaiac (see below) and (5) 20 to 30 drops of old turpentine. If blood be present the mixture turns to a blue-violet; if absent, a red-brown, often with a tinge of green. The reaction is more plain if some water is added and the color extracted with chloroform.

Cowie⁴⁵ has modified Weber's test by adding an equal amount of distilled water to the acetic acid ether extract, and thoroughly shaking, before adding the guaiac, and claims for this method a greater delicacy than any other modification of Weber's test; *e. g.*, Weber, by guaiac, obtained a positive reaction for blood in stools after ingestion of 3 gm. of blood, while Cowie was unable to verify this by Weber's test, yet with his "water modification" he was able to get a *distinct reaction after the ingestion of 0.5 gm. of blood*. Cowie, like Boas, adds a few granules of powdered guaiac to the ether extract, instead of preparing the customary guaiac tincture; when the guaiac is dissolved, the oil of turpentine is added.

In the comparative tests given below, the following method was employed:

1. About 5 ccm. of liquid gastric contents or 3 gm. of feces (the feces should be thoroughly mixed and made fluid by rubbing up with a little water) are mixed with one-third the quantity of glacial acetic acid.

2. Extract with about 10 ccm. of ether. To avoid the emulsifying action of ether, instead of shaking the tube, it should be slowly inverted several times.

3. *A piece of clear brown gum-guaiac about the size of a cherry is crushed and then dissolved in a test tube half full of alcohol (or ether, see below). This tincture should assume a light sherry color. (Green oxidized portions must not be used.)

4. To perform the test, about $\frac{1}{2}$ ccm. of the guaiac tincture is added to about 5 ccm. of the acetic acid ether extract and to this 1 ccm. of a 3% peroxide of hydrogen (the usual commercial strength) or an equal amount of old oil of turpentine. (The H_2O_2 may be added to the tincture of guaiac first, when, if the tincture is strong, it may turn milky, but this is clarified on addition of the ether extract. This procedure serves as a control on the guaiac.)

In the presence of blood, a blue color quickly develops, which deepens, and in two or three minutes gradually fades to a pale blue and disappears; in the case of feces the disappearing color is sometimes a faded green. In fecal extracts, also, various shades of purple, purple-brown or red-brown frequently replace the blue, and with small traces of blood even a green shade. This green shade may never result from pure blood solutions, but I am convinced this sometimes occurs with fecal extracts, as I have seen cases where only a green shade occurred with the guaiac when the benzedin and paraphenyldiamin tests were clearly positive. As much difference of opinion prevails as to what colors, other than shades of blue, result from the presence

of blood, shades of green should not be considered positive when a more delicate test is negative. When peroxide of hydrogen is used two layers form and the color reaction appears in the upper ether layer. H_2O_2 seems to give a somewhat quicker reaction and Boas found it more delicate. Oil of turpentine may mix with the ether extract, the color appearing throughout the mixture, but it usually settles to the bottom where the color appears. It was suggested by Schmilinsky¹⁰ (p. 2146), and recently by Cowie,⁴⁵ that a color scale may be arranged so that the guaiac test could be used quantitatively, but the writer believes that color changes can only be used in a rough and general way to estimate the quantity of blood, and, as Clemm¹¹ (p. 377) states, such a scale is inconstant and not worth the tax on the judgment.

Should an emulsion form in making the ether extract it can be broken up, as pointed out by both Schmilinsky¹⁰ (p. 2146) and Schumm² (p. 19), by adding a few drops of alcohol and giving the tube a few turns. The latter author claims that Boas's⁷ warning that alcohol itself may cause color changes cannot be accepted, as in 1,000 applications of the test he used strong alcohol many times for this purpose and never noted this occurrence.

If the stools contain much fat it is better first to extract with ether, as recommended by Boas and Rossel,⁹ before making the acetic acid-ether extract, as the guaiac reaction is thus made clearer. (There is no danger of losing blood, as hemoglobin in a neutral or alkaline medium is not absorbed by ether.) If there is much hydrobilirubin or vegetable coloring matter present, the writer has found the guaiac test much improved by first extracting with alcohol-ether (equal parts), instead of ether, which removes not only the greater part of the free fat and fatty acids but much of the coloring matter as well but not of the blood. (See also Schumm's method below.) Boas, White and Cowie believe the fat extraction is not often necessary.

When turpentine is used as the oxidizing agent it should be perfectly pure and clear (as Mercks) and before ready for use should be exposed to the air in an open dish and in a dark place for at least a month; afterward it should be kept in a bottle uncorked, or only lightly protected with cotton. It should not be left in strong sunlight, for Schumm² (p. 20) has seen turpentine thus treated give an intense blue with the plain tincture of guaiac, and I have seen such a specimen give a tinge of green-blue with guaiac.

Guaiac resin for testing purposes should be clear brown or wine color. A large piece 4 or 5 inches through (if possible) should be obtained, and a clear piece broken off when needed for use. Any oxidized (green) portions should be thoroughly removed, as these without the presence of blood may give color reactions; and for this reason guaiac, for testing purposes, should never be kept in the powdered form, as every particle becomes oxidized and untrustworthy for blood tests. Guaiac may be dissolved in either alco-

* As the following tests were begun by Weber's old method it was continued throughout, but the method used by Boas of adding a few grains of fresh guaiac directly to the ether extract and then adding the oxidizing agent is fully as delicate and more simple.

hol or ether. It dissolves more readily in alcohol and is better for immediate use—it should be prepared about every hour. If a solution of guaiac is to be kept on hand, an ethereal tincture must be made, as this remains active for months.

The writer has not found the ether solution as satisfactory as the fresh alcoholic tincture, and the green residue which forms around the rim of the bottle may be a source of error in the former. The best method, as already mentioned, is to add a few bits to the ether extract with each test.

It is customary, before testing for occult blood in the stools, to place the patient on a special meat-free diet. Sometimes, however, it is important to make occult blood tests without any change of diet. It is thus necessary to know how much and what kinds of meat will usually give a positive reaction.

It is generally stated that red meats* in moderate quantities, eaten once daily, will sometimes give positive guaiac, and that cooking, though it does not prevent, lessens this reaction, and also that fish, fowl and ham do not give a positive test even if eaten in considerable quantity. Eggs never interfere with the test.

I have often examined stools of patients who had been on a mixed vegetable and meat diet, but never obtained a positive reaction by Weber's test in any case in which blood was absent. This agrees with Cowie's statement (p. 412) that an ordinary meat diet seldom if ever causes a reaction for occult blood. Cowie (l. c.), however, obtained a clear, positive guaiac reaction after the ingestion of 10 gm. of scraped raw meat, but no reaction when smaller amounts than 10 gm. of scraped raw meat had been ingested. (Further meat experiments under benzedin.)

As to the action of the guaiac test on crude drugs, the various preparations of iron, etc., probably little can be added to what was stated by Schönbein and van Deen and Liman 50 years ago; but the acetic acid ethereal extract of the stools prevents all drugs, probably, from interfering with the guaiac test for blood, and Weber³⁰ (p. 443) himself found of 67 medicines administered in various combinations to patients, that the acid ether extract of stools of these patients gave no action with the van Deen test. By the same method stools gave negative results after ingestion of milk; the ether acetic extract removed cause of error in presence of milk, pus, saliva or spices; and greens or bile caused less confusion when van Deen's test was performed by Weber's method.

The blue shade one obtains by the action of the guaiac test on Bland's mass and on the tincture of chloride of iron very quickly disappears, though it is very like that produced by old blood. When Bland's mass is treated with acetic acid, water added and shaken with ether, the ether extract gives no reaction with guaiac. I have many times applied Weber's test to stools which were black through administration of Bland's pills, the dose often being 30 gr. daily, and in the absence of blood no positive action was ever

obtained. Steele and Butt¹⁴ found, also, that ferric carbonate, 30 gr. daily, and ovoferrin and peptomangan in daily doses of 1½ oz. failed to give a reaction similar to blood by Weber's test. It is safe to consider that no drug in therapeutic doses will give a reaction by Weber's method which may be confounded with blood. The delicacy of guaiac as indicated by various writers is given in the following:

		In 1 gm. of Stools * with Blood Content of		gm.
		+ guaiac	0.0008	
Boas & Kochman				
Schloss (p. 275)	+	"	{ 0.0008	"
			{ 0.0006	"
Hartman (p. 51)	+	"	0.00005	" to 0.00001
Cowie (p. 419)	+	"	0.00035	"
Clemm (p. 377)	+	"	0.001	"
Schmilinsky (p. 2146)	+	"	0.01	"

+ equals positive.

* Or stomach contents.

Schloss (l. c.) was unable to confirm Hartmann's results either with guaiac or aloin, though Cowie⁴⁵ (p. 422) is inclined to believe the results of all correct. Schumm and Remstedt⁵² (p. 993) obtained a barely positive guaiac solution of blood in water of 1 to 100,000; when 8 ccm. of the solution was used.

Schumm's² (p. 26-29) modification of Weber's test is performed in this way: About 4 gm. of feces (portion, size of walnut) is thoroughly rubbed up in a glazed porcelain mortar with 30 ccm. of alcohol-ether (equal volumes); the contents are then transferred to a clean coarse filter placed in a funnel (the filter should be unplaited and about 12 cm. in diameter); the residue in the cone is again treated with alcohol-ether (30 ccm.) and assisted in running through by stirring with a glass rod; when the filtration is completed some plain ether is poured over the filter and stirred as above, and when the ether has filtered off the residue is washed once more with ether. This process, in a neutral or alkaline mixture, usually removes the coloring matter, neutral fats and free fatty acids (soaps are not removed by neutral alcohol-ether), and it likewise removes the water present. Four cubic centimeters of glacial acetic acid are next added to the residue on the filter and gently but thoroughly worked through by means of the glass rod. Then 4 ccm. more of acetic acid are added and the stirring repeated, and when a greater portion of the acid has drained off, the combined acid filtrate is again poured over the cone and allowed to thoroughly filter. A number of cubic centimeters of the acetic acid filtrate is diluted with two or three times its volume of ether, and to this is added a half volume of distilled water, and the mixture shaken. If separation does not occur, add some alcohol, or ether, or water. The acetic acid ether extract is now tested in a thoroughly clean and dry † or alcohol-washed test tube. In the presence of hematin there appears in a short time a more or less clear violet-blue, red-violet or green-blue. Another portion of this extract may be used for spectroscopic examina-

† Cowie⁴⁵ insists on dry test tubes, as both he and his assistant, Dr. Roche, have obtained a positive guaiac (clear blue) when dry test tubes were used, and a negative test with the same specimen when tubes were not dry.

* See experiments under benzedin.

tion. In the case of liquid stools the procedure is the same, only a larger amount is taken and stirred up with about four times the quantity of alcohol-ether. If the stool is acid in reaction it is first treated with a number of drops of concentrated sodium carbonate solution, and then with the alcohol-ether.

Schumm claims great delicacy for his method. The writer has made few comparative tests with this process, but in the cases where tried, the colors were not only more clear and intense than with the guaiac test by Weber's method, but likewise when other test-agents, as aloin or benzedin, were used. The respective color changes were greatly enhanced when an extract of the specimen was made as directed by Schumm. Were it not a time-robbing procedure, Schumm's method would meet about all the requirements for a clinical test.

ALOIN.

Aloin was first described as a blood test by Klunge⁴⁶ twenty-five years ago, later by Rossel⁴⁷ and then by Boas,⁴⁷ Koziezkowsky,⁴⁸ von Torday,⁴⁹ Petracchi⁴¹ and others.

For a time it seemed that aloin might displace guaiac because of its greater delicacy and reliability. In the past two years, however, it has been less recommended and some recent writers, as White and Cowie, have concluded from their experience that it is probably less delicate than the guaiac. Koziezkowsky obtained a positive aloin reaction in a mixture of 0.025 gm. of blood with 2 gm. substance and Cowie⁴⁵ (p. 415) obtained positive aloin with 0.02 gm. blood to 1 gm. of feces, and a questionable reaction with 0.005 gm. to 1 gm. of feces.

The preparation of the material for the aloin test is in all particulars the same as for the guaiac test. Oil of turpentine is better than H_2O_2 , inasmuch as the reaction is more clear. To prepare the aloin solution, a portion of the powder (a good preparation, and the one used in these tests, is that of Parke Davis) is placed in a *clean* dry or alcohol washed test tube—sufficient to fill the bottom of the glass—and to this is added about 10 ccm. of 75% alcohol (aloin is scarcely soluble in ether, only about 1 to 500); when the solution becomes yellow it is ready for use. The test is then performed throughout as with guaiac, using the same proportions of reagents. Aloin solution should be made fresh, as after standing about half an hour it usually assumes a pink or slightly red color which is increased on addition of old turpentine.

The color change in a positive reaction is a pink or slight red to deep cherry red, or, in extract of stools, a red wine color. The color reactions are not as prompt as with guaiac and in the presence of only small amounts of blood the red color is not distinct for ten or twelve minutes. If the color only appears after fifteen minutes or more it should not be considered positive, as this sometimes occurs in the absence of blood. By adding a drop or two of chloroform the appearance of the color is hastened.

When the aloin solution, ether extract and oil of turpentine are mixed, by lightly shaking, the aloin solution usually sinks to the bottom where the color changes occur, but sometimes, as pointed out by Koziezkowsky (p. 1199) and Steele, the mixture becomes uniform, particularly if the aloin solution is made with strong alcohol (95%).

Ninety-eight occult blood tests were made with aloin and compared with an equal number of guaiac tests performed as suggested by Weber. Fifty-one of these comparative tests were made in stomach contents, and in three of these cases, in which the guaiac test was negative, the aloin test gave slight red color in five to fifteen minutes, and in each of these three cases the phenyldiamin test was positive. On the other hand, in a case of hyperchlorhydria with free HCl 0.26%, the guaiac test gave a slight blue, while the aloin test gave only a very slight pink, not distinctly positive (in this case the paraphenyldiamin was positive and likewise the benzedin). In another case the guaiac test gave a slight *red*, the aloin a slight pink in about twelve minutes, which did not increase, and the paraphenyldiamin was slightly positive. In one specimen of urine the guaiac test was doubtful (slight green tinge), a pink color appeared with the aloin and the paraphenyldiamin was positive. In the other urinary tests the guaiac and aloin agreed, except in one specimen in which the guaiac test (slight tinge of blue) as well as the phenyldiamin were positive, the aloin was completely negative. In two specimens out of forty-seven stools the guaiac test was negative, while the aloin gave a slight pink and the phenyldiamin was positive; in the remaining tests there was no discrepancy between the aloin and guaiac tests. The above results indicate that the guaiac (Weber's modification) and aloin tests are about equally delicate. (Further comparisons under phenyldiamin and benzedin.)

PARAPHENYLDIAMIN TEST.

A new and very delicate reagent for detection of occult blood has recently been introduced by Boas.⁵⁰ It was originally employed by Storch⁵¹ in detecting pasteurized milk. The reagent consists of a very dilute solution (about 1 to 200) of paraphenyldiaminechlorhydrate with the addition of peroxide of hydrogen. With unheated milk the reagent gives an indigo blue, and the whey, as it separates, shows a violet and brown; in pasteurized milk the test gives a blue-gray color.

This test with certain modifications has been found by Boas a reliable and more delicate test for occult blood in feces than guaiac or aloin, but not so sensitive as benzedin. He states that for months he used it exclusively as a blood test, but believes the other methods should be retained as control tests in cases of doubt.

To perform the test according to Boas the stomach contents or feces are prepared much as with the aloin and guaiac tests, except that a smaller amount of acetic acid, about 20 drops, is added to the contents and extracted with ether as in the guaiac test; to 2 or 3 ccm. of this ether extract add 1 or 2 drops of a very dilute solution

(1 to 100) of a paraphenyldiaminechlorhydrate; to the mixture is added 1 ccm. of a half normal alcoholic potassium hydrate solution, and then 10 to 15 drops of a 3% H_2O_2 . Upon slight shaking there appears almost momentarily in the bottom of the reagent glass a characteristic olive green (or blue-green) color, but especially characteristic is the olive green ring between the layer of ether and peroxide of hydrogen; the upper layer shows a more or less violet color. The green color is very transitory and, according to the blood content, quickly changes into a more or less deep violet. In the absence of blood the green color is either very faint or does not appear at all. If the stools are deeply tinged with bile or hydrobilirubin, they should first be extracted with alcohol-ether.

Following the directions of Boas, I applied this test to 103 specimens of gastric contents, stools and urines, at the same time making comparative tests with both guaiac and aloin, and in 43 of the specimens additional comparisons with benzedin. In no specimen where I obtained a positive reaction with either Weber's or the aloin test did I fail to get some degree of reaction with the phenyldiamin. In 11 of the cases where the latter was positive, Weber's test was negative, and in 7 of these the aloin test was negative. In a majority of these 11 cases the transient green ring, which Boas terms "extraordinarily characteristic," was absent, and in such cases a diffuse light green tinged with yellow appeared, quickly changing to a weak dirty brown or washed-out violet. I have considered as negative those reactions of phenyldiamin where no green shade was discernible in the beginning of the color changes. In stomach contents less discrepancy was found between the results of this test and those of aloin and guaiac than was the case in feces. Almost invariably when the phenyldiamin gave a positive reaction with gastric contents, the guaiac gave a clear blue and the aloin a distinct red.

The phenyldiamin test was never positive when the benzedin was negative, but it was negative six times when the benzedin was positive.

Schumm and Remstedt⁵² have recently modified the procedure as carried out by Boas in the following way:

To the fluid under investigation are added only 2 drops of a 1 to 200 solution of paraphenyldiaminechlorhydrate, then 1 ccm. of about one half normal alcoholic potassic hydrate, then 1 ccm. of a 3% peroxide of hydrogen, and last of all are added a few drops (about five) of a dilute acetic acid. A newly prepared watery solution of freshly defibrinated human blood tested in this way shows in a moment an olive green color which gradually turns to brown red. In such a solution these writers found this test to be somewhat more delicate than the guaiac, and claim they were able to determine blood when in a watery dilution of 1 to 100,000 when 8 ccm. were used; on the contrary, if the acetic acid was added before the potassium solution the test failed in a blood dilution of 1 to 40,000 — no greater than the delicacy of the guaiac test, determined long ago by van

Deen.⁵¹ Schumm and Remstedt⁵² never obtained a positive reaction in the stools by means of this test when Schumm's modification of Weber's test was negative or only suggestive. I have made no systematic comparison with this modification of the phenyldiamin test, but the test is delicate and it might be preferred to Boas's method, if only on account of the readiness with which the color changes are detected at the top of the solution. There is no ring formation, but in very minute quantities of blood a light green color almost immediately appears and gradually diffuses toward the bottom of the tube, and in the meantime the various shades of violet (as described above) have begun to appear. Schumm and Remstedt (p. 994) mention that in weak dilutions of blood the olive green may not appear, but only a red color. This is easily demonstrated in a watery solution of blood.

BENZEDIN TEST.

Benzedin was first described as a color test for blood by O. and R. Adler⁵³ in 1904, and is the most delicate of all the clinical tests for occult blood. Of the many chemical combinations (amido bodies, phenols, amido acids, naphtholine and diphenyl groups) with which they experimented, they found that benzedin, of the diphenyl group ($\text{H}_2\text{N}-\text{C}_6\text{H}_4-\text{C}_6\text{H}_4-\text{H}_2\text{N}$), in a saturated alcoholic solution, and with the addition of peroxide of hydrogen, determined the presence of blood in a dilution of 1 to 100,000 (0.001%). Schumm, however, found the limit of delicacy 1 to 200,000, when he used 8 ccm. of a fresh watery dilution of human arterial blood. It would detect 0.00004 gm. of blood. The guaiac test under equal conditions he found required eight times this strength of blood solution to give a reaction (detecting only 0.00032 gm. blood). He claimed the delicacy of the reaction depended upon the quality of the benzedin used; he employed Mereks "benzedin purissimum," but he had obtained *commercial preparations of "benzedin puriss," which were not so delicate as the guaiac test.* Schumm's results are easily verified. I have found that fresh human blood (hemoglobin 100%) in a dilution of 1 to 200,000, by volume, gives a clear pale green, with an acetic acid solution of benzedin and H_2O_2 in one minute, turning to a pale red in 8 to 10 minutes. If the blood solution is first heated to boiling and the benzedin reagent added while the blood solution is hot the color changes are very rapid, almost instantaneous, but the green color is not so evident, and the red shade appears within half a minute. If the blood is first treated with glacial acetic acid or HCl and then diluted, the color changes seem not so distinct as with pure blood solutions, yet gave a positive benzedin in a dilution of 1 to 175,000. Boiling the acetic acid blood solution lessens the power to cause color changes. Siegel,⁵⁴ and Schlesinger and Holst,⁵⁵ and Schumm,⁵⁶ also review the subject of benzedin and report their investigations, but Schlesinger and Holst materially modified the test recommended by A. and O.

Adler and gave it a distinct clinical value, which the original test did not possess.

The Adlers⁵³ employed benzedin as follows:

I. For urine, 10 or 15 ccm. are treated with a half volume of acetic acid and extracted with ether. On adding to the ether extract some saturated alcoholic solution of benzedin, with peroxide of hydrogen, the usual green color appears in the presence of hematin.

II. For feces, a small quantity is mixed with some water and to 3 ccm. of the unfiltered mixture is added 2 ccm. each of freshly prepared benzedin solution and peroxide of hydrogen, and three or four drops of glacial acetic acid. The presence of blood causes an intense green.

These authors, although they point out that certain oxidized ferments (indirect oxydases) in the presence of peroxide of hydrogen can be destroyed by boiling, do not mention this procedure in their directions.

Schumm² (p. 14) in applying the test to feces, according to this method found the reaction too delicate for clinical work.

Schlesinger and Holst, by modifying the original test, overcame the extreme sensitiveness and yet kept it beyond the delicacy of all other tests for occult blood in feces. Their method is as follows:

I. An approximately saturated solution of benzedin is made by adding a knife-point full of benzedin to about 2 ccm. of glacial acetic acid in a clean test tube; shake and set aside; the benzedin soon becomes dissolved. The solution is better if made fresh, though it is active after many days.

II. A piece of feces about the size of a pea is stirred up with a clean glass rod in a test tube about a fifth filled with water (test tube about 10 cm. long). The tube is then lightly closed with a stopper and the mixture brought to a boiling point over the flame. (The boiling destroys the activity of the enzymes.)

III. About 10 or 12 drops of the concentrated acetic acid solution of benzedin (I) is now poured into a clean test tube and 2½ ccm. to 3 ccm. of a 3% solution (ordinary commercial) hydrogen peroxide. A reagent is thus prepared which will not only serve as a control on the chemicals, but likewise on the reagent glasses.

IV. To perform the blood test we pour into the reagent (III) one to three drops of the boiled feces mixture (II) after lightly shaking it.

In the presence of blood there develops a beautiful green, blue-green or blue. The greater the blood content the more predominant is the blue.

The appearance of the color-change in a case where the aloin test would be positive occurs in a few seconds, while in the presence of only a slight amount of blood ¼ to ½ minute is required. The time varies, but Schlesinger and Holst²⁸ state, and it is easily demonstrated, that even in the case of minute traces of blood the beautiful green color always appeared within two minutes. (Further description of color changes in Table I a.)

Following the modifications of Schlesinger and Holst²⁸ I have made 107 applications of this test (stomach contents, feces and urines), nearly all of which were in comparison with the aloin, guaiac and phenyldiamin tests. (In all the experiments I used the benzedin purissimum of Merck, Germany.) It is also important that the H₂O₂ should be pure, as I have found two preparations of peroxide of hydrogen which invariably gave a green color when added to the benzedin solution alone. "Dioxygen" of the Oakland Chemical Company and H₂O₂ of Parke Davis do not cause a green color with a pure solution of benzedin in glacial acetic acid.

In 36 of the specimens (gastric contents and feces) all four tests were applied to each specimen, making in all 161 applications (as in 17 of the specimens the benzedin test was applied both before and after boiling). In 12 of these series the results were uniformly negative with all the tests, five of the specimens were stomach contents, 7 were stools, obtained when the diet had been meat-free for at least three days. (These results demonstrate the importance of a negative benzedin test, and the amount of time it is possible to save in routine work where benzedin is applied first, as the whole test, including the preparation of materials, can be performed within two minutes.) In the gastric contents of one case, where the benzedin and phenyldiamin were distinctly positive the aloin was negative, but the guaiac showed a slight green developing in less than a minute, then disappearing; but in two specimens of stools the benzedin, phenyldiamin and aloin were positive, while the guaiac was negative. In one of these cases the aloin became a light red wine color at the end of 15 minutes, in the other a slight red in 10 minutes, and in this case the aloin and guaiac tests were repeated three times.

In 32 specimens of gastric contents the benzedin test was negative in 16. In 6 of this series the test was *positive before* boiling and *negative after* boiling the contents. This may show that enzymes * as secreted and under certain condi-

* In connection with these examinations of the gastric contents, the benzedin test was applied to the following preparations:

Preparation.	Age of Specimen.	How Treated.		Benzedin Test.
From one manufacturer:				
Scale and powdered pepsin.	Three years old.	Not boiled.	—	
Scale and powdered pepsin.	Fresh from manufacturer.	Not boiled.	—	
Pancreatic powder.	Fresh from manufacturer.	Not boiled.	+	
Pancreatic powder.	Fresh from manufacturer.	Boiled.	—	
Pancreatic powder.	Fresh from manufacturer.	Not boiled.	—	With acetic acid ether extract.
Essence pepsin.	Fresh.	Not boiled.	—	
From another manufacturer:				
Peptonizing powder (from tubes)	Fresh.	Not boiled.	+	Deep green blue.
Peptonizing powder (from tubes)	Fresh.	Boiled.	+	Distinct green.
Peptonizing powder (from tubes)	Fresh.	Not boiled.	+	Distinct clear green with acetic acid ether extract.
Peptonizing powder (from tubes)	Fresh.	Boiled.	—	With acetic acid ether extract.

tions give a positive benzedin. In these cases the green color reaction was usually very light, however, and slow in appearing, though usually within two minutes. The tests were carried on side by side and six drops each were used of boiled and unboiled contents. When 1 ccm. or 2 ccm. of boiled contents are added to the reagent a light green may develop in cases where 2 or 3 drops cause no color change. The test may be negative in *unboiled* gastric contents not only when HCl is absent, but when the free HCl content is high.

In 22 specimens of stools 10 benzedin tests were negative and the remainder positive. No definite green color change was found *before* boiling that was absent *after* boiling the specimen, but in nearly all the 10 negative tests color change was wholly absent without boiling, proving that enzymes seldom, if ever, interfere with the benzedin test in stools.

Stools which are suspected of containing blood, but give a negative blood test, should be well mixed and again tested, as a stool may contain portions *passed at the same time*, one of which will give a strong positive blood reaction by all blood tests, and another portion give a negative result with the benzedin as well as all the other tests. Such an occurrence was illustrated in the case of a patient (a woman fifty-two years old) seen with Dr. J. B. Thornton, and presenting a group of symptoms which suggested gastros-taxis* or gastro-enterostaxis. At first the stools were composed of soft and unformed masses, dark blue, or black, then becoming tarry, or

very dark. On the fourth day, after sudden cessation of all symptoms, the patient passed a stool which gave both negative and positive blood tests. In this formed stool one portion was brown and gave an intense positive reaction by aloin, guaiac, phenyldiamin and benzedin. Other parts were light yellow (as seen after milk diet), and portions taken from the center of these light colored masses repeatedly gave a negative result with the benzedin, and with all the other blood tests. In some of the masses there had been a slight admixture, giving positive benzedin reaction and perhaps a slight reaction with some of the other tests. This seemed to be such an unusual occurrence that a great many tests were made at different times (and with care) for confirmation. Why the benzedin test could be negative in such a case as this, when, as has been stated by Grünwald²⁸ (p. 110), a positive test with benzedin was obtained in the stools $2\frac{1}{2}$ days *after ingestion* of a ham sandwich, seems quite inexplicable. Schlesinger and Holst,²⁷ however, obtained by their method a negative benzedin reaction in stools in one case 60 hours and in another 36 hours following the ingestion of meat, though it was always positive within 24 hours, and in one case it gave a positive reaction 44 hours following the ingestion of 0.5 gm. of blood.

Experiments 1 and 2 indicate the results of the benzedin and guaiac tests when applied to normal stools, after the ingestion of various foods, and the increase in delicacy of either test when applied to the fecal extracts prepared according to Schumm.

EXPERIMENT No. 1.

Day.	Food Eaten.	Time of Stool.	Adler's Benzedin test with Schlesinger's and Holst's Modification.	Benzedin with Schumm's.	Weber's Test.	Schumm's Modified Weber's.	Aloin with Schumm's Extract.
21st and 22d.	Hearty evening meal of very rare roast beef for two days, other meals meat free.	No examination of stool on these days.					
23d.	Food during day: 3 eggs, salt codfish and cream, potato, bread, butter, oatmeal, tea, coffee.	Morning stool.	Strongly positive, specimen unboiled. Positive, specimen boiled. (a).		Positive (b).		
24th.	Eggs, oatmeal porridge, cream, milk, bread, butter, coffee.	Morning stool.	Positive, unboiled. Positive, boiled.	Intensely (c) positive.	Negative.	Positive (d).	Positive (e).
25th.	Oatmeal porridge, 1 egg, fish cakes, fresh mackerel, cream, milk, bread butter, coffee.	Morning stool.	Positive, unboiled. Negative, boiled.		Negative.	Negative.	Negative.
26th.	Breakfast, oatmeal porridge, few baked beans.	Morning stool lost. Stool at 3 P.M.	Negative, unboiled.	Strongly positive (f).	Negative.	Negative.	Negative.

Preparation.	Age of Specimen.	How Treated.	Benzedin Test.
Holladin.	Fresh.	Not boiled.	+
Holladin.	Fresh.	Boiled.	+
Protonuclein.	Fresh.	Not boiled.	+
Protonuclein.	Fresh.	Boiled.	—
Takadiastase.	Fresh.	Not boiled.	—

It is difficult to show that some of the + reactions with these specimens were not due to blood. We should also expect a positive reaction to any considerable amount of enzyme powder that was active *in vitro*, but this was not the rule; and it may be that the enzymes which yield a positive reaction with benzedin are not always present or active in the commercial preparations.

* H. Wale White: The Lancet, London, Nov. 3, 1906, p. 1189. *Idem.*, Jan. 12, 1907, p. 126.

(a) Color change, intense green in three quarters minute, in one to two minutes green changing to black, in three minutes black color has changed to claret-red and no further change in fifteen minutes. On adding more feces solution, claret-red returns through black to green, back through black to red. With the boiled specimen, the same changes occurred as above, only the colors were much less intense.

(b) The color change was a light green with red brown in one half minute, green and red disappeared and left a brown which faded. (c) The color change was a clear and intense green with the ether acetic acid extract (Schumm), and with the straight acetic acid extract (Schumm) a very intense green blue.

(d) The color change was a suggestion of green, quickly followed by a dark red brown, but when the straight acetic acid extract was used (without ether extraction) the color was a clear blue.

(e) The color change was a tinge of red in five minutes with the ether acetic acid extract (Schumm), but on using the straight acetic acid extract a clear red wine color quickly appeared.

(f) Color change was a green blue with the straight acetic acid extract (Schumm) and a clear deep green with the ether acetic acid extract (Schumm).

EXPERIMENT No. 2.

On the 8th, evening meal, — cold roast beef, the other meals, meat-free; on the 9th, evening meal, — beef pie, other meals meat-free; on the 10th, eggs, lobster, salt codfish and cream; on the 11th, eggs and asparagus (otherwise on 10th and 11th meals were meat-free). Stools passed on 8th, 9th and 10th not examined. Examination of stools on 11th, thirty-two hours after meal of meat pie, showed both the benzedin and Weber's tests negative. In the case of benzedin the feces solution was unboiled. The stool was then extracted by Schumm's method and both the benzedin and guaiac tests were found clearly positive. Stool passed on 12th, fifty hours after meat pie gave a negative guaiac by Schumm's and only a slightly positive benzedin. Although salt fish fiber gives a positive benzedin the experiment shows it does not cause a positive reaction after digestion.

I have applied the benzedin test (method of Schlesinger and Holst) to various substances with the following results: Raw green vegetables gave a slight positive reaction, though it was not found that they materially interfered with the test, in gastric contents or stools, and particularly in the latter. Raw wheaten flour, corn and oatmeal, potato, carrot, parsnip and squash, all gave a quick positive reaction; when cooked the result was negative with all. All meats, cooked and uncooked, gave a strong positive reaction. Raw fish, fresh, dry and pickled, and shell fish (oysters and clams) gave a positive reaction with benzedin; cooking much weakened the reaction, and the color change was chiefly confined to the tissue substance, the solution showing only a weak green. Cooked lobster, not mixed with the visceral portions, showed no benzedin reaction. After the ingestion of any form of fish the feces usually caused no benzedin reaction, sometimes slightly positive after fresh fish. Eggs, raw or cooked, did not give a positive benzedin. The presence of biliverdin in the urine or feces caused a very strong positive reaction, but bilirubin, hydrobilirubin (or urobilin) as found in the stomach contents, feces or urine, either gave no benzedin reaction at all or only a slight degree of color change.* Bland's pills give a quick but not intense positive reaction with benzedin, but after ingestion of Bland's pills (30 gr. daily), the feces gave no positive reaction with benzedin (by Schlesinger and Holst's method).

The requirements of any clinical blood-test are accuracy, delicacy and simplicity of application. All of the occult blood-tests embrace one or two of these requirements, but Teichmann's and the spectroscopic tests have already been considered

as generally inferior to all others. Taking *guaiac* as a standard, the above comparative tests show the following:

The *aloin* test is accurate and was found about equal in delicacy to the *guaiac* test, by Weber's method; if, however, a slight distinct tinge of green or a red brown is taken to indicate blood, then *guaiac* will be found the more delicate test. At first one is likely to be impressed by the singleness of color change in the *aloin* test; but, on the other hand, the *aloin* is slow of action, particularly in the presence of small amounts of blood, and, furthermore, we do not have the choice between H_2O_2 and oil of turpentine, — it requires turpentine. The *aloin* test holds no distinct advantage over *guaiac*.

The *paraphenyldiamin* test (Störch-Boas test) was frequently found on comparison more delicate than Weber's, and is probably more delicate than any modification of the *guaiac* test, except possibly Schumm's. Still, the test is not so simple as the *guaiac* test, and the varying color changes are confusing, and, in examining stools, bile and other coloring matters, are as likely to interfere as in the *guaiac* test. Apart from its delicacy, I can find nothing to recommend the *paraphenyldiamin* over the ordinary *guaiac* test.

Benzedin is by far the most delicate of the occult blood tests. In fact, the objection has been repeatedly raised that it reacts to such minute quantities of blood as to be clinically useless. The modification of Schlesinger and Holst overcomes the objection of excessive delicacy of the test, and renders it more practical and exceedingly simple (it can be performed within two minutes) and also prevents any possible reaction from enzymes. But even this modification may not prevent small amounts of blood (even non-pathological) from giving a slightly positive benzedin sometimes, but practice soon teaches what degree of color change indicates occult blood.

Frequently the benzedin test is negative, and it is for this reason of great value, obviating the need of other tests, and this is particularly true as a test for feces.

A positive benzedin and a positive *guaiac* would be more trustworthy than a positive benzedin alone, and *guaiac* is a *safer* test than benzedin for stools where meat has *not* been excluded from the diet.

Benzedin and *guaiac* together meet every practical demand of a clinical test for the presence of blood.

I am much indebted to Dr. Pratt for important suggestions in the subject-matter of this paper, as well as for facilities offered in his clinic.

* I am much obliged to Dr. W. W. Barker, Medical House Officer, Massachusetts General Hospital, for a number of specimens supplied for these tests.

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A CRITICAL ESTIMATE OF THE FERMEN- TATION SPECIFIC GRAVITY METHOD OF QUANTITATING SUGAR IN DIABETIC URINE.*

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In 1861 Roberts¹ described a method of quantitating the glucose present in diabetic urine, which he spoke of as "the clinical method," on account of its simplicity and ease of application. His method consisted in determining the decrease in specific gravity of the urine caused by the action of yeast. Yeast fermentation splits up the glucose present and its amount can be determined by multiplying the difference between the specific gravity of the urine before and after fermentation by 0.23. This gives the percentage of glucose present. Roberts compared the results obtained by this with those from other methods of that day for quantitating glucose, and showed the considerable accuracy of his method.

Of late years the method suggested by Roberts has been rather neglected. In its place titration with some form of copper sulphate solution, polari-

*Read in the Section on Medicine of the American Medical Association at the Fifty-eighth Annual Session, held at Atlantic City, June, 1907.

¹Edinburgh Med. Journ., 1861, vii, 326.