On the guaiacum process for the detection of blood in medico-legal cases : the antozone test / by Alfred S. Taylor.

ON THE

GUAIACUM PROCESS

FOR THE

DETECTION OF BLOOD IN MEDICO-LEGAL CASES.

THE ANTOZONE TEST.

By ALFRED S. TAYLOR, M.D., F.R.S.

In the spring of 1867, I received from Dr. John Day, of Geelong, Australia, a report of a case in which he had succeeded in detecting blood on articles of clothing under circumstances of great difficulty.

"On the 19th October, 1866, a murder was committed at a place named Scarsdale, and a Chinaman was taken up by the police on suspicion. The trousers he wore at the time he was apprehended had recently been washed, but there were some slight stains on a part of them, and a small piece was cut out and sent to Mr. Johnson, the Government analytical chemist, for examination. In his evidence he stated he had failed by chemical tests to recognise blood on the cloth sent to him by the police, in consequence of the quantity being so minute, but that he had discovered a slight trace of blood by the aid of the microscope. At my request he very kindly sent me the identical piece of cloth on which to try my test. It was accompanied by a note, of which the following is a copy:

"'St. Kilda; *November 27th*, 1866.

"'Dear Sir,—With pleasure I enclose a portion of the very cloth cut out from the Chinaman's trousers on which I detected

blood by aid of the microscope, not without some trouble and long search. If your test is superior to those known, it may, perhaps, act in the present case. I shall be glad to know what it is, and how you succeed.

"'Truly yours,

(Signed) "'Wm. Johnson.

"'J. Day, Esq., M.D., Geelong.'

"On the day I received the piece of cloth, the blood-stains, if from the murdered man, would have been thirty-eight days old. It was rather dirty, but there was no blood to be seen on it by the unaided eye. I succeeded, however, in the course of a few minutes, in striking off sixty impressions from the cloth, each impression showing bright blue spots wherever blood-globules were present; after the sixtieth impression, the blue spots were difficult to produce and almost invisible, and before I got to the seventieth impression, the blood-globules seem to have been all destroyed and the reaction ceased.

"The mode of using this blood-test must of course depend on the nature of the material on which blood-stains are supposed to be present. The plan I adopted in applying it in the case of the Chinaman's trousers was as follows:—I first poured a few drops of tincture of guaiacum over the cloth, and then a drop or two of ozonized ether. The blue colour did not show on the cloth, but on putting a slip of white blotting paper on it, and gently pressing it with an ivory paper knife, I got a perfect impression, and then another, and so on until all the blood-globules were destroyed. All that was necessary was to add a little more ether, or perhaps a little more guaiacum. In testing for blood on white materials there is, of course, no necessity for striking off impressions on white paper."

Dr. Day forwarded to me a small portion of the trousers referred to in the above communication. The material was a close cotton fustian of a dirty brown colour. I could not by the aid of the microscope detect upon the stuff any stain or spot resembling blood, nor could I extract any red colouring matter from the stuff by cutting it into small fragments, digesting them in water, and afterwards compressing them. A part of the fibre, which was somewhat stiffened, was wetted with tincture of guaiacum,

which produced no change of colour in the stuff, and peroxide of hydrogen was afterwards added. The blue colour which is caused by the red colouring matter of blood under these circumstances was not apparent on the brown fustian, but on pressing the wetted spot on white blotting paper two faint blue impressions were obtained; thus corroborating Dr. Day's results some months after the performance of his experiments.

The application of a solution of guaiacum for the detection of blood stains is not new. It is some years since it was first suggested as a method of research in medico-legal inquiries, by Van Deen, a Dutch chemist, but the process appears to have attracted but little attention until 1863, when Van Deen's experiments were made the subject of a minute critical examination by Dr. Liman, of Berlin.[1]

Van Deen employed an alcoholic solution of guaiacum prepared with the pure resin, and oil of turpentine containing what he supposed to be ozone, the liquid commonly described as "ozonized" oil of turpentine. Dr. Liman performed fifty-three experiments on a variety of substances with these liquids, applying them to fresh and old blood in the liquid state, as well as to dry stains produced by blood on clothing and weapons, and noting accurately the results. The general conclusions at which he arrived may be thus summarized: 1. When a negative result is obtained by the process, it might be justly concluded that blood was not present. 2. When the reaction gave a positive result (a blue colour) it could not be affirmed that blood was certainly present, without some additional corroboration. If the stain had the usual colour and appearance of blood, although diluted with water,—if a watery extract of an unstained portion of the stuff did not give the reaction, while the extract from the stained portion did produce the blue colour, then it might be fairly inferred that the stain had been caused by blood. These qualified conclusions were calculated to convey the impression that the process was, on the whole, not trustworthy. Some experiments which I performed by Van Deen's process in 1864, led me to some extent to adopt Dr. Liman's views,[2] and it was not until after receiving the communication

[1] "Neue Versuche zur Erkennung von Blutflecken, und zur Prüfung von Van Deen's Blutproben." Casper's 'Vierteljahrsschrift,' B. 24, 1863, p. 193.

[2] 'Principles and Practice of Medical Jurisprudence,' 1865, p. 2.

above mentioned from Dr. Day that I was induced to revise the whole of my former experiments, and in place of oil of turpentine to employ the liquid suggested by Dr. Day, namely, ozonized ether, which was subsequently laid aside for peroxide of hydrogen.

These experiments have satisfied me that oil of turpentine is not a favorable liquid for producing the results. It does not readily mix with water or alcohol, and there is some difficulty in determining whether it really contains sufficient ozone, or rather antozone for the purpose. Some of my former unsatisfactory results I attribute partly to the use of an impure alcoholic solution of guaiacum, and partly to the use of a non-ozonized or imperfectly ozonized oil of turpentine. Dr. Day has improved Van Deen's process not merely by suggesting the use of ether in place of the oil of turpentine, but by enabling the operator to determine in it by a very simple method the presence of antozone. The greater number of the objections made by Dr. Liman to this process are referable to the action of a variety of substances upon guaiacum resin alone, but they are in great part removed by the fact that the colouring matter of blood produces no change in guaiacum, except in the presence of antozone.

It will be understood from the preceding remarks that the guaiacum process for the detection of the red colouring matter of blood, depends on the use of two liquids: 1. A solution of that portion of guaiacum resin which is dissolved by alcohol (·830); and 2. A liquid containing, not ozone, as Van Deen supposed, but antozone or peroxide of hydrogen, as demonstrated by the experiments of Schönbein and Dr. Day. A saturated solution of guaiacum should be made, the inner portions of the resin which have not been changed in colour by air or light being selected for this purpose. The solution should be preserved from light by being kept in a bottle covered with black paper, or in a dark closet. When a few drops of this solution are added to water there is a milky-white precipitate of the resinous principle in a state fit for oxidation. The precipitated resin exposed to air very slowly acquires a blue colour as the result of absorbing oxygen. Light alone appears to have no effect upon it. I have exposed some of the freshly precipitated resin to a strong light for many months in a hermetically-sealed tube, and it has preserved

the same white opaline tint which it had when first produced. So far as I could judge, light did not accelerate the oxidation of the precipitated resin,—for two portions equally exposed to air, one being placed in the strong light of a window, and the other in a dark closet, underwent a similar colouration after the same period of exposure.

When the precipitated resin is added to a jar of oxygen gas, and well shaken, it becomes more rapidly blued than in air, but the most remarkable change is produced by adding the precipitated resin to a jar containing ozone or an ozonized atmosphere. The resin acquires almost immediately an intensely blue colour. These facts show that the blueing of the resin depends on a change of colour produced by oxidation. As an additional proof of this it may be stated that all those hyperoxides which, according to Schönbein, contain a portion of their oxygen in the form of ozone, and are called by him ozonides, possess the property of blueing the resin directly in a well-marked manner. One drop of a solution of manganate or permanganate of potash renders the precipitated resin immediately blue. The peroxides of lead and manganese produce a similar change in it, but more slowly. Other bodies which operate through the agency of water as oxidizers produce a similar result. Thus the resin is blued by solutions of chlorine, bromine, and iodine, by hyponitric acid, and by the hypochlorites. Mineral and organic compounds also possess this property in different degrees, and although we may infer analogically that the resin is oxidized by nascent oxygen (ozone), it is not easy to see in all cases whence the oxygen is derived. The persalts of iron exert a strong blueing action, and here, perhaps, the salt undergoes partial deoxidation; the ferrocyanide and ferricyanide of potassium, and even finely divided platinum (platinum black), render the precipitated resin blue. As platinum black decomposes iodide of potassium, setting iodine free, it is not improbable that it may contain ozone, and that this is the secret of its powerful oxidizing action on bodies, the fine state of division merely increasing the surface of contact.

Among organic substances we find that gum, gluten, and unboiled milk render the guaiacum blue, while starch, fibrin, boiled milk, and the red colouring matter of blood produce no change of colour in it. A cold solution of gum acacia very slowly

blues the precipitated resin, while gluten operates more rapidly, producing in a short time a well-marked blue colour. Milk which has not been boiled blues the resin slowly; after boiling even for a short time, it causes no change of colour in it. The blue colour which the resin acquires from contact with the pulp of raw potato, and the juices of many fresh roots, which have not been exposed to heat, may be owing to the presence of gum gluten, or vegetable fibrin. As in the animal liquid (milk) heat destroys the property, so among vegetable substances heat also prevents the least reaction. Thus the pulp of raw potato renders the resin blue, but when boiled, it loses this property. So with regard to gum acacia, a cold solution blues the resin, but a solution made with boiling water and cooled, does not change the colour. Platinum black strongly heated and added to the precipitated resin when cold still preserves the property of blueing it rapidly.

As in all these experiments the mixture of resin and water is exposed to air, it may be assumed that oxygen is transferred either directly from the mixture to the guaiacum resin, or that a mixture of certain substances with guaiacum causes a rapid absorption and transference of atmospheric oxygen. One fact, however, is certain—the property is sometimes destroyed by a very moderate heat, and is not restored to the substance on cooling.

It follows from what has been stated that the blueing of guaiacum is in all cases a simple process of oxidation, and that it may take place sooner or later by the mere contact of many mineral and organic substances with the freshly precipitated guaiacum resin.

A large number of substances are without any action upon the resin. Starch, fibrin, and albumen do not render it blue, and among animal liquids the red colouring matter of the blood and boiled milk do not in any way affect it. It is further remarkable that those mineral compounds which, according to Schönbein, contain oxygen in the form of *antozone*, as the peroxide of hydrogen, peroxide of barium, and the peroxides of the alkaline metals generally, exert no oxidizing action on guaiacum resin, and do not blue it. The guaiacum resin therefore, as Schönbein pointed out some years since, is well adapted to distinguish an ozonide from an antozonide. Both

oxidize the iodide of potassium and set free iodine, but it is only the ozonide containing negative oxygen which renders the guaiacum blue. The antozonide containing positive oxygen has no such effect.

Peroxide of hydrogen added to the precipitated resin produces no change of colour in it. Peroxide of barium (an antozonide) produces at first a yellowish colour (owing to the presence of some baryta), but on adding a drop of acetic acid to correct this, the liquid is colourless and white. Liquids containing oxygen in the state of antozone, act in a similar manner. Thus the varieties of ether, sold as "ozonized" ether, whether a product of ethylic or methylic alcohol,—"ozonized" essential oils, as of turpentine or lavender, do not change the colour of the resin, and it is therefore clear that they contain no ozone. Other facts show that they contain oxygen as antozone. The name given to these ethers, therefore, is based on a mistake. They resemble the peroxide of hydrogen (antozone), and differ from the ozonides, not only in this want of an oxidizing action on guaiacum, but also in the remarkable property first pointed out by Schönbein, namely, of converting the red chromic into blue perchromic acid.

If a few drops of a solution of bichromate of potash, acidified strongly with dilute sulphuric acid, are added to a small quantity of peroxide of hydrogen, and the mixture is well agitated, a soluble compound of an intense sapphire blue colour is produced. This is the perchromic acid of Schönbein ($Cr_2 O_7$). On adding a small quantity of ether it is dissolved by this liquid, and rises with it to the surface. Ethylic or methylic ether, in what is termed an ozonized state, produces a similar blue compound with the acid bichromate, which is at once dissolved in these liquids. It is thus demonstrated that they contain peroxide of hydrogen or antozone, and not ozone. No ozonide will produce perchromic acid under these circumstances. It is not, however, easy to produce this conversion with any antozonide, excepting peroxide of hydrogen and the ethers holding it in solution. If this experiment is performed with ozonized alcohol, spirit of lavender, eau-de-cologne, or other essential oils dissolved in spirit, the chromic acid is converted into oxide of chromium. Oil of turpentine is probably in the same condition as the other essential oils. It does not blue the guaiacum, and does not

contain ozone. Its oxidizing power on iodide of potassium and the bleaching of indigo are most probably due to the presence of antozone. It produces no perchromic acid on the addition of bichromate of potash, and thus the antozone cannot be easily detected in it. It is well known that peroxide of manganese added to peroxide of hydrogen decomposes this liquid, and sets free ordinary oxygen. So in reference to these "ozonized" ethers, it is found that peroxide of manganese produces in them a similar change. Crystals of permanganate of potash dropped into antozonized ether set free oxygen as when dropped into peroxide of hydrogen, thus furnishing an additional proof that the oxygen contained in the ether is in the form of antozone. From the complete insolubility of the permanganate in oil of turpentine, the crystals appear to undergo no change when placed in this liquid.

Ether which contains antozone acquires immediately a dark bluish or greenish colour on the addition of acid bichromate of potash, while that which does not contain antozone is merely coloured yellow. The essential oils and alcoholic solutions of them may contain antozone, but there is no ready method of applying a test to determine its presence in them. The bleaching of indigo by essential oil of turpentine is a rough method of testing, since it must depend greatly on the quantity of blue colouring principle present and the quantity of the oil added.

The red colouring matter of blood, whether dissolved in water or alcohol, whether recent or of many years' standing, whether taken from a mammalian animal or from a bird, fish, or reptile, does not oxidise or render blue the freshly precipitated resin ~~of~~ of guaiacum. I have thus tried fresh human blood, and human blood which had been kept in a bottle for twenty years in the liquid state, also the blood of the sheep, ox, pig, rabbit, pigeon, pheasant, carp, herring, and the frog. The result was the same, —according to the quantity added, the resin acquired a slight reddish colour, but no blueing took place.

The guaiacum process for the detection of the red colouring matter of blood, therefore, rests upon the simple fact that the colouring principle in all red-blooded animals has no direct oxidizing or colouring action on the resin; but when associated with another body containing antozone, equally without any

oxidizing action on the resin, the guaiacum is oxidized by the blood, and acquires a blue colour varying in intensity according to the amount of red colouring matter present. Precipitate the resin by adding a few drops of the tincture to four drachms of water. Divide this liquid into two portions. Add to one a small quantity of an aqueous solution of the colouring matter of blood, enough to give the faintest red tint, and to the other add a few drops of a solution of peroxide of hydrogen.[1] There will be no change of colour in the resin in either glass, *i. e.*, neither the blood nor the peroxide (antozone) will oxidise the guaiacum or turn it blue. If to the first glass containing blood and resin, a few drops of peroxide are added, a blue colour begins to show itself in a minute or two, just as if a liquid containing ozone (a solution of permanganate of potash) had been added to it. If the quantity of resin precipitated is large compared with the quantity of red colouring matter of blood which is present, the blue colour may be so concealed as not to show itself distinctly until several minutes have elapsed. On the other hand when the red colouring matter of blood is in excess, the colour produced will be of a dingy indigo or dirty violet. In all these cases, however, there is an easy method of bringing out the colour. The oxidized resin is soluble in alcohol, retaining its blue colour. On adding sufficient alcohol to dissolve the precipitated resin, which renders the liquid turbid, it will become clear and the alcoholic solution will acquire a deep sapphire blue colour. If there is much albumen associated with the red colouring matter, this will of course remain undissolved.

If to the second glass, containing a mixture of resin and peroxide, a solution of blood is added, the same result takes place, and the intensity of blue tint according to the quantity of blood added, may be accurately observed. It is of little importance, therefore, so far as the mere results are concerned, which of the two liquids is first added; but as the guaiacum resin is liable to be coloured from oxidation by the direct contact of many substances in the absence of peroxide of hydrogen, it is always desirable in order to avoid any fallacy, to add the suspected liquid to the resin before the peroxide. If a blue or greenish colour is thereby produced, although blood may still

[1] To be procured of Robbins & Co., 372, Oxford Street.

be present, there is some oxidising substance in addition to blood which may conceal its presence. If the aqueous solution of blood has been boiled so as to coagulate and entirely destroy the red colouring matter, this process will not detect it. The guaiacum resin and peroxide will undergo no change of colour when placed in contact with it.

It is a singular fact that the red colouring matter, as it is extracted by digesting the dried coagulum in boiling alcohol, produces with the tincture of guaiacum and the peroxide, a blue coloured liquid, the alcohol in this case being sufficient to keep the resin in solution. Hæmine crystals obtained by a somewhat complex chemical process also acquire a blue coloration when treated with the guaiacum and peroxide of hydrogen. Being insoluble they become simply changed in colour from a dark red cinnabar tint to blue. During the last summer, Dr. Iwan Gwosdew, of Moscow, who attended the course of lectures on medical jurisprudence, assisted me in a variety of experiments on this subject. Some very pure hæmine crystals, which he had prepared by a process of his own, were tested and found to retain in this altered condition the property above mentioned.[1]

It will be understood that under proper precautions this process simply enables the operator to say whether that which he is examining is the coloring matter of a red-blooded animal. It throws no light on the class of animal to which the blood belongs, —it may be hot-blooded or cold-blooded, a mammal or a reptile —and under no circumstances will it enable the examiner to solve that question which so frequently arises, namely, whether the blood is human or from a mammalian animal. I have even found the red fluid resembling blood from the body of the common house-fly (*musca domestica*) to produce this change in guaiacum with peroxide of hydrogen.

So again the process does not enable the examiner to detect the blood-globules or cells or even to speak of their presence. The test applies only to the red colouring matter removed from the globule or cell. Thus the test is applicable even when the cell is completely destroyed by water or alcohol, and the colouring matter is in such small proportion as barely to tinge the water. Yet with a proper adjustment of the proportions of precipitated

[1] 'Uber die Darstellung des Hämin aus dem Blute,' von Dr. Iwan Gwosdew. May, 1866.

resin and peroxide of hydrogen, the blood-red will be revealed by a blueing of the guaiacum.

The colouring principle of blood thus diffused in water does not possess the properties of an ozonide. Thus, it does not blue guaiacum resin; it does not oxidize the potassium and set free iodine from the iodide, and it has no bleaching properties on indigo. These facts show that it does not contain ozone.

How then does the red colouring matter of blood act under these circumstances? The answer to this question is not very obvious. It operates by converting the antozone of the peroxide of hydrogen into ozone or nascent oxygen. This theory was first promulgated by Schönbein, and is generally adopted; but why the coloring matter of blood of all red-blooded animals should possess this property, while it is not found associated with animal or vegetable liquids and solids, or with other organic red-colouring matters is not apparent. Thus, pure starch diffused in water like blood has no action on guaiacum, and the addition of peroxide of hydrogen produces no change. Milk which has been boiled for a short time is without any action on the precipitated guaiacum: on the addition of peroxide of hydrogen the resin remains unchanged. Herein consists the special value of the guaiacum test, whatever theory may be adopted to explain the facts; with red blood the addition of peroxide of hydrogen causes oxidation or blueing, but with other organic liquids and solids, which like red blood are without action on the resin, the addition of the peroxide creates no difference. The resin is not oxidized, and does not acquire a blue colour. It is equally difficult to explain why milk unboiled and a solution of gum acacia unboiled should oxidize guaiacum, while they lose this property as a result of boiling. The most delicate ozonoscopic paper impregnated with iodide of potassium and starch, fails to show the slightest trace of ozone in milk or a solution of gum acacia or in a diluted solution of blood; but the latter, when mixed with peroxide, instantly oxidised guaiacum.

The organic red colouring principles which might be mistaken for blood have no action on guaiacum like that above described. The colouring principle of cochineal in water was mixed with a diluted solution of albumen, so as to render it as nearly as possible similar to blood. When guaiacum resin was added to this mixture it was simply reddened. A small quan-

tity of peroxide of hydrogen was then added. It underwent no change of colour; it still remained red. The red colouring principles of fruits, flowers, leaves, and woods—red wine, red ink, the red of the rose, kino, catechu, the colouring matter of Brazil wood, safflower, and other red colouring principles which might be mistaken for blood were tested with similar negative results. They in the first instance redden the precipitated guaiacum resin, and when peroxide of hydrogen is added there is no change of colour. They cannot, therefore, be mistaken for blood. The admixture of blood with them is on the other hand indicated by a change of tint when peroxide of hydrogen is added; the blue mixing with the red produces a dingy violet tint which is deep in proportion to the amount of blood present.

In the above remarks I have considered this method of testing as applied to blood in the *liquid* state. It is always desirable, if possible, to obtain the red colouring matter dissolved in water. Articles of clothing, furniture, or weapons may present spots or stains which are said to be dried blood. By cutting up the cloth or scraping the dry substance from the weapon and macerating it in a small quantity of distilled water for some hours, we may by pressure obtain from the stuff a liquid of a reddish or reddish brown colour. The solubility of the red colouring matter in water is one of the characters of blood, and it is thereby distinguished from spots of iron-rust (iron moulds), red dyes fixed by mordants, red paint, and other red mineral colours. If no soluble colouring matter is thus obtained it is probable that the stain is not owing to blood; if the water is coloured, we should allow time for the subsidence of dirt, iron rust, and insoluble matters, and then pour off the liquid portion. A quantity equivalent to ten drops, or the sixth part of a teaspoonful is quite sufficient for testing. The colour when very pale may be best seen by examining the liquid through a long narrow tube. The guaiacum test may be then applied. When this test is applied for the first time, the small quantity of red colouring matter which it admits of detecting, creates surprise. Blood so diluted as barely to give a stain to white blotting paper thus admits of detection, and thus it is that the process is admirably adapted for tracing blood in articles of clothing which have been washed or soaked in water for the purpose of effacing the stains. So small a quantity as one drop of blood in eight ounces of

water may be thus detected by operating on one or two drachms.

The small quantity which may be detected by this process is beyond the application of the ordinary chemical tests, namely, ammonia and the effect of heat. The microscope shows nothing, for all the globules or cells have been destroyed by water, and the colouring matter simply is diffused through the stuff in minute quantity. There is only one other process which can compete with it in delicacy, and that is Mr. Sorby's method of examining the liquid by the spectroscopic eye-piece attached to the microscope, and noting the position of the two dark absorption bands in the green portion of the spectrum. By an ingenious arrangement the suspected liquid can be at the same time examined and compared with human or animal blood diluted in an equal degree. Traces of blood mixed with urine, mucus, or other liquids which have no oxidising action on guaiacum with or without the peroxide of hydrogen, may be thus readily detected. In the use of the spectroscopic microscope the colour of the liquid is intensified by viewing it through a section of a barometer tube about an inch in length, half an inch in thickness, and one eighth of an inch in the bore. A visible colour is thus obtained with the characteristic spectrum of blood from so small a quantity as six to eight drops of a liquid, which but for this mode of examination, first suggested by Mr. Sorby, would appear colourless and would give no spectrum. In comparative experiments on these processes, I have found that in all cases in which the two absorption-bands were rendered visible by the ingenious arrangement above mentioned, the guaiacum readily acted and produced in the pale liquid the characteristic blue colour indicative of blood. Liquids such as cochineal and ammonia, which by the spectroscopic process ~~might~~ give absorption-bands somewhat resembling those of blood, ~~are~~ clearly distinguished from blood by the guaiacum test, for ~~they~~ produce no blue coloration of the resin. Blood in all stages of change is indicated by the guaiacum test, while for the spectroscopic process blood in some stages of change requires a peculiar treatment with certain chemicals before the absorption bands are rendered visible. For most purposes, the two processes may be regarded as equally delicate, and it is fortunate that they admit of being used with the same quantity of coloured fluid, the guaiacum

being applied after an examination of the liquid by the spectroscopic microscope.

It may be naturally supposed that two such delicate processes leading to the detection of blood in the most diluted condition, are likely to fail under similar circumstances. Thus it is indispensable to the action of both that a coloured liquid should be obtained. If a stained cloth has been boiled in water or washed in boiling water, so as to destroy completely the red colouring matter, neither process will reveal the presence of blood thus altered by heat. Again, under the most favorable circumstances, the spectroscopic method carries the investigation no further than the guaiacum process. It enables the operator to detect the red colouring matter of red-blooded animals; it takes no note of the globules or cells; and the absorption-bands in the green portion of the spectrum have a similar position whether the blood is that of a human being, a mammalian animal, a bird, fish, or reptile. Contrasting in this way the blood of man, of the rabbit, pheasant, pigeon, herring and frog, I could perceive no difference. There is also another condition in which in accordance with Mr. Sorby's experience as well as my own, the spectroscopic process will fail. This is in those cases in which the blood has been washed in a diluted state into clothing, and the colouring matter thus spread over a large surface. The diluted blood exposed to air for many weeks or months appears to undergo some change which renders it impossible to extract it by water in a condition to admit of the characteristic spectrum being produced. Mr. Sorby informs me that he made two examinations of a portion of the Chinaman's trousers, referred to at the commencement of this paper, but did not succeed in procuring from them a liquid which gave the spectrum of blood. In the hands of Dr. Day, when the stains were five weeks old, a number of blue impressions were produced from them by the aid of guaiacum, and some were obtained by myself five months afterwards by the same process. This difference arises from the fact that the guaiacum process will yield its results by direct application of the two liquids to the stained cloth, while for the spectroscope a liquid with some slight shade of red must be obtained from the cloth in an independent form.

These facts do not detract from the value of a beautiful

optical process so skilfully worked out by Mr. Sorby, of Sheffield, but for the purposes of medical jurisprudence and the rigour of practice, it is right that we should know what our processes will really prove and what they will leave unproved. The kind of red-blooded animal from which the blood has been taken, the sex, the age, the period at which it escaped from the blood-vessels, and whether arterial or venous, are points which are still unsolved by science. These two processes of research have not removed our difficulties in this respect; but they have enlarged our means of speaking with certainty to the presence of small quantities of blood upon which a scientific chemist but a few years since would have hesitated to give an opinion.

Blood-stains on clothing.—In examining articles of woollen or silk, dyed black, or of a dark colour, some care is required or spots of blood may be easily overlooked. In general the stuff is stiffened by the drying of the albumen. When examined with a low power of the miscroscope the stain of blood may appear, if recent, glossy and smooth on the surface,—dry coagula enveloping the fibres of the stuff, and in certain lights a crimson tint may be seen which is peculiar to blood, and with which the eye by practice may soon become familiar. The stain may be sufficiently large to be cut out with a penknife or scissors. In this case the stained stuff may be cut into small fragments and macerated in a covered watch-glass with distilled water. In a few hours the liquid thus obtained may be poured off, and if coloured, examined by the spectroscope, and subsequently by the guaiacum process, or the latter only may be at once applied.

The search for corpuscles or cells must be made in the ordinary way, and with a high power of the microscope. A portion of coagulum, if obtainable, should be placed on a glass slide moistened with water containing a little glycerine or iodide of potassium, and covered with a plate of thin glass until a red liquid begins to appear at the margin. At this time the globules may be sometimes seen in the act of escaping from the coagulum.

The stain on dark woollen or silk may be so small and so imbedded in the stuff as not to admit of removal by water. In this case the plan suggested by Dr. Day is admirably adapted to detect blood. The spot is first wetted with a little water; a drop

of tincture of guaiacum is then allowed to fall on it. It should now be pressed firmly on white blotting paper, and if no blue stain is produced there is nothing in the dye or material or in the blotting paper to affect the application of the test. Another drop of tincture of guaiacum is then added to the stain, and this is followed by two or three drops of a solution of peroxide of hydrogen. No change of colour may be observed, but in a minute or two a blue impression of the blood-stain may be procured by firmly pressing the wetted portion of the cloth on white blotting-paper. Blood may be thus readily detected on dark articles of clothing in which the colour of the woollen renders it difficult to see any stain, and the shape and form of the spot are sometimes pretty clearly indicated by the form of the blue stain produced. This remark applies also to stains of blood on woollen which have been sponged or washed for the purpose of obliterating them. Unless all the red colouring matter has been removed, which is an extremely difficult process, blood may be detected by the direct application of guaiacum and peroxide of hydrogen. If the woollen is grey or light coloured the production of a blue colour may be seen at once on the stuff. If it is thick woollen it is probable that the red colouring matter has been washed to the inside, and has spread by imbibition. It may be there detected, and the boundary of the washed portion determined by repeated applications of the guaiacum and peroxide at different parts. Before any conclusion is drawn, however, a portion of the clothing should be similarly treated on which there can be no suspicion of blood having fallen, and which presents no appearance of staining. The results should, of course, be negative. As a rule, this experiment should be first performed, because it furnishes good negative evidence that the guaiacum process may be safely applied directly to the stained article of dress. It is not probable that all parts of a woollen garment should be stained with blood, hence this comparative experiment may be performed without difficulty. To articles of woollen, silk, cotton, or linen which are not coloured, the guaiacum test admits of an easy application. In November, 1857, a towel was stained with a number of spots of blood, and in some parts with bloody water. In the present month (December, 1867), *i.e.*, after the lapse of ten years, these stains were examined and tested. They had a dark red-brown colour, no lustre,

and no distinct appearance of coagula or dried clot to the naked eye. A small spot of undiluted blood was wetted with water and tincture of guaiacum then dropped on it. No change of colour was observed; the peroxide of hydrogen was then added, and a deep blue stain made its appearance in the situation of the stain. The intensity of the blue colour was increased by the addition of a few drops of alcohol. A similar experiment was performed on a well-washed stain of blood in which the red colouring matter was so diluted as scarcely to tinge the towel. A light blue colour was produced by the test in one or two minutes, and this was intensified by the addition of alcohol. The tint produced under these circumstances varies from a deep indigo blue, where the red colouring matter is abundant, to a pale azure blue, where it is at a minimum and scarcely visible to the naked eye. The blue colour thus produced, if moderately strong, will remain for weeks or months without material change. Neither light nor air appears to have any decomposing effect upon it. A corner of the same towel was now selected as being quite free from any stains of blood or bloody water, and the stuff was here treated in the same manner as the stained portions. The guaiacum and peroxide became dry without producing any visible change of colour. There was no blood on this part of the towel. The affirmative and negative results obtained in these experiments showed conclusively that dry blood in its ordinary state, and washed blood in a most diluted state, may be easily detected by this process after the lapse of *ten years*. The towel in this case had been lying loosely exposed in a drawer which was frequently opened.

Fruit-stains.—These create no difficulty. On colourless articles of clothing they present either to the naked eye or when seen through a lens an equal and superficial staining wholly unlike blood in colour. There is no stiffening of the fibre, no appearance of clot, and the addition of a weak solution of ammonia may impart to them either a greenish, an olive colour, or a crimson tint. The colouring matter of a blood-stain undergoes no change by the addition of weak ammonia. The guaiacum and peroxide applied to fruit-stains produces no blue colour; hence, if no change of colour took place, the inference would be that the stain was not owing to blood. If, however, a blue colour

was produced, it should be noted whether this is caused by the guaiacum only, and, further, whether an unstained portion of the colourless stuff does or does not produce a similar colour with the test.

Iron stains.—Iron moulds.—These, when old, have an ochrous or ruddy brown colour. On cotton and linen they are commonly observed to penetrate equally both sides of the stuff. When examined with the naked eye, and still better with a lens or a low power of the microscope, they are quite different from stains caused by blood either in a diluted or undiluted state. There is no appearance of fibrin or coagulum, no glossiness of surface, no stiffening of the stained fibre, and an absence of anything approaching to the crimson or red tint of blood. Water will not dissolve or diffuse the stain; but a mixture of equal parts of water and hydrochloric acid will speedily dissolve it, especially by the aid of heat,—the iron mould, as it is called, disappears, and the acid liquid now holds a salt of iron, which may be discovered by all the usual tests.

If tincture of guaiacum is added to an iron-mould on cotton or linen it undergoes no change. The peroxide of iron is in a perfectly insoluble form, and it has no action on guaiacum resin. The iron mould remains equally unchanged by the addition of the peroxide of hydrogen to the guaiacum. There can, therefore, be no difficulty in distinguishing stains of blood from iron-moulds; but it may be desirable to demonstrate that the stain is really caused by peroxide of iron. The iron-stain, wetted with tincture of galls, undergoes no change, owing to the insolubility of the oxide. It should be first wetted with glacial acetic acid and dried by a gentle heat. The stain is not thereby removed, but the iron is partly converted into acetate, and if, when dry, a drop of the tincture of galls is added, the stain at once acquires a dark purple colour. Its ferruginous character is thus indicated.

Ink-stains.—Stains caused by ink, owing to their peculiar colour, are not likely to be mistaken for stains of blood so long as they are on colourless articles of clothing; but if such stains are upon black cloth, silk, or woollen, and the guaiacum test is applied directly to the stuff a fallacious result might be obtained. Ink contains a persalt as well as a protosalt of iron. Tincture of guaiacum added to very diluted ink, produces a

mixture which becomes rapidly blue by oxidation. All the salts of the peroxide of iron, including the sulphocyanate, operate in a similar manner, and at once render the guaiacum blue. A very minute quantity of perchloride of iron will also cause this change in guaiacum. The addition of any form of antozone is not necessary to produce this change of colour with the salts of iron, and it does not in any way increase it. Herein, then, lies the distinction. If the stain is on black cloth the guaiacum solution should be added, with a little water to dissolve the ink, and the wetted stuff firmly pressed on white blotting-paper. If ink is present there will be a blue spot produced, showing that the stain is probably owing to a salt of iron. The cloth, cut into fragments and macerated in distilled water, will yield a dark purple or bluish-black liquid, having the usual appearance of diluted ink, and wholly unlike blood. The guaiacum solution added to this aqueous liquid, is precipitated, and the precipitated resin rapidly acquires a blue colour, without the addition of peroxide of hydrogen.

But iron may, in some instances, be present without being indicated by a stain or discoloration in the substance. Thus white or tawed leather produces a blue colour with guaiacum and peroxide of hydrogen, very like that caused by blood: it also produces a blue colour, but more slowly, with guaiacum alone. Alum and salt are used in the manufacture of this leather, and as when sold for manufactnring purposes these substances frequently contain a notable quantity of iron, the blueing effect on the guaiacum may be owing to the presencę of this metal thus transferred to the skin. Some kinds of white kid leather are open to the same observation: hence blood stains on white kid gloves should, if possible, be removed by water, and the red aqueous liquid separately tested. Some kinds of writing paper sized with sulphate of alumina containing iron, produce spots of a blue colour with the guaiacum. When flour paste has been used in the dressing of the paper, the guaiacum may be rendered blue by the gluten, which is incorporated with the pulp. Filtering paper and calico are occasionally charged with flour-paste and in this case the guaiacum might be coloured by coming in contact with gluten.

Stains on weapons.—None but those who have frequently had occasion to examine weapons can be aware of the difficulties

which sometimes present themselves in determining whether reddish-brown stains on knives, razors, hatchets, hammers, &c., are owing to blood or rust. Some kinds of rust on these articles so resemble dry blood that I have known even experienced surgeons to be deceived. In fact, no safe reply can be given to the inquiry in the absence of experiments. In dealing with articles of this description the same principles should guide the operator as in the application of the guaiacum process to clothing. If any dry coagulated blood is on the blade or in the indents of the letters, or in the joint of the handle, it should be scraped off and digested in a watch glass with a few drops of distilled water. If a coloured solution is obtained, this should be separated by decantation or filtering from any iron rust, and it may then be tested by guaiacum in the manner described. If the blade of the instrument has been washed or the blood remaining on it is merely in a thin film, as the result of wiping, a solution sufficient for the guaiacum and spectroscopic processes may still be procured by placing the flat side of the knife on a thin layer of water on plate-glass. After a time, if any blood is present, the water will acquire a colour, and it may then be poured off and tested.

If the deposit on the weapon consists of iron rust alone, no red colour will be imparted to the water, since common rust is quite insoluble, and no blue colour will be produced on the addition of guaiacum and peroxide of hydrogen. The deposit will be found quite soluble in strong hydrochloric acid, forming yellow perchloride of iron and giving all the reactions for iron with the usual tests. A portion of the dry rust scraped from the iron may be placed in a watch glass, and after being moistened with water, a small quantity of guaiacum and peroxide added to it. If it be rust unmixed with blood, there will be no change of colour. Any particles of dry blood will slowly acquire a blue colour. In many cases we find blood and rust associated. Unless the weapon has been thoroughly washed some coagulated blood may be found in the indentations of the letters of the maker's name. If it has been recently washed and not wiped dry the marks of light orange coloured rust may be found in the inner portions. Old rust is indicated by its dark red or red-brown colour. In all cases the instru-

ment should be taken to pieces, since some blood may have penetrated between the blades or the layers of the handle.

Certain kinds of rust are soluble in water, thus the rust caused by the vegetable acids, citric, acetic acids, &c., is of a yellowish- or reddish-yellow colour and water will dissolve a portion of it. The solution is of a pale yellowish colour: it contains a soluble persalt of iron. It blues guaiacum without the peroxide, and has all the usual reactions of iron. Although, as seen on the weapon, it might resemble dry blood, it could not be mistaken for it when dissolved by water.

In these experiments I have advised the use of peroxide of hydrogen as the source of antozone. Dr. J. Day of Geelong has used ozonized ether. The principle is the same in the two cases. The ozonized ether owes its properties to the peroxide of hydrogen. Dr. Day sent to me, from Australia, samples of various ozonized liquids, which he had employed in his experiments.—1. A sample of ozonized ether, which had been nine years in the colony; 2, ozonized oil of lavender; 3, ozonized eau-de-cologne; 4, methylated sulphuric ether: No. 1 acted with guaiacum on blood almost as rapidly and as strongly as the peroxide of hydrogen itself. It contained much antozone. The other liquids, as well as some old spirit of lavender which I had by me, produced similar results, but more slowly.

In order to see how far this theory of the action of guaiacum and an antozonide on the red colour of blood was correct, I performed some experiments with the peroxide of barium. This is a solid antozonide. As it has been elsewhere stated, it does not render guaiacum blue: but when a small quantity of the colouring matter of blood is added, the resin, with which it is mixed, acquirs a blue colour as when mixed with other antozonides. This is not, however, a convenient form in which to employ an antozonide. We have, therefore, now to consider which of the liquid antozonides is preferable for use. Applying the correct names, the operator must employ either antozonized ether—antozonized oil of turpentine, or antozonized oil of lavender, dissolved in alcohol. There may be other essential oils equally or more effective, but this is a matter for further investigation. Apart from the actual testing by means of the colouring matter of blood, there is an easy method of determining whether the ether is in a proper state for experiments

namely by the addition of chromic acid (see page 437), and the production of perchromic acid. This mode of testing is ~~inapplicable~~ to the essential oils, and no good practical method has been suggested by Van Deen or others, to distinguish that kind of oil of turpentine fitted for the experiment from that which is not fitted. The nature of the oil renders it ill adapted for experiments in colouring matters dissolved by water. Nevertheless a good sample of the antozonized oil of turpentine may be made to produce all the reactions described in this paper. I found that the antozonized oil applied to old stains of blood (of ten years' date) which had been moistened with tincture of guaiacum, produced in the red coloured spot a deep indigo-blue colour; in the washed portions it produced a light azure blue; but I did not find it so delicate a reagent for detecting blood diluted with water as the antozonized ether, or peroxide of hydrogen. I shall only remark here that some injustice has been done by medico-legal writers to this process of Van Deen. It has been pronounced untrustworthy, because there are many substances which impart a blue colour to guaiacum resin. The statement is true, but it does not convey the whole truth. The real question upon which the value of the guaiacum process must rest in its medico-legal application, is this—What red colouring principles are there which are soluble in water, and will not render guaiacum resin blue, except in the presence of an antozonide? In the large number of experiments performed by Dr. Liman he appears to have used the guaiacum and antozonide together. In this case the results would, of course, be the same with blood and a great variety of substances. But by this method of operating the fact is concealed, that blood does not act on guaiacum, except when an antozonide is present, while the other substances equally act in its absence.

If it is antozone or peroxide of hydrogen which confers this property of acquiring a blue colour on a mixture of blood and guaiacum, it would seem reasonable to employ this at once and discard the use of ether and essential oils, which are at the best but solvents of unknown quantities of this peculiar compound. My results with pure peroxide of hydrogen are the same as those announced many years since by Schönbein—namely, that it does not oxidize or render guaiacum resin blue.

Nevertheless, samples of that which is sold as pure peroxide do not always come up to this standard. I have found the solution of the peroxide to contain, sometimes sulphuric, and sometimes hydrochloric acid, and to have varying degrees of acidity. Some acid is generally added for the purpose of preserving it. Although this may not affect its properties for medicinal use, it may affect the results when it is employed with guaiacum as a test for blood. The solution should not give a precipitate with nitrate of silver or chloride of barium, and when added to a small quantity of tincture of guaiacum in a tube kept corked, the precipitated resin should not acquire a blue or green colour. Some samples of peroxide thus mixed with guaiacum have acquired slowly a greenish blue tint, and the resin has been separated in a curd by the acid present in the liquid. In using antozonized ether and oil of turpentine I have not observed this change of colour. In a closely corked tube the precipitated resin remains white. I believe that the colouring effect sometimes produced by a solution of peroxide of hydrogen may be owing to the admixture of an acid (hydrochloric), containing iron. A very small quantity of the perchloride of iron in solution is sufficient to produce the change of colour in the guaiacum. In reference to the peroxide, if it gives a copious precipitate with nitrate of silver, it should be rejected, and in all cases in which the peroxide is used, the rule laid down should be strictly adhered to—namely, in applying it to stains on clothing—the two liquids should always be applied to an unstained portion of the same clothing. In testing the colouring matter of blood dissolved in water, equal parts of a mixture of guaiacum (precipitated) and peroxide should be placed in two glasses, and the blood-liquid added to one of them. The difference in the results as to colour will then be at once seen.

With respect to tincture of guaiacum, it appears to lose its property when long kept, probably as a result of exposure to light and air at the same time. Tinctures which give a deep reddish-coloured precipitate with water are commonly unfit for use. The best test for determining whether the guaiacum is in a proper state, is a small quantity of the colouring matter of blood mixed with an antozonide. The result of this experiment will show whether the liquid is in a proper state for use or not. The resin should become blue in the manner described.

As a summary of these observations on examining stains of blood on clothing or weapons it may be stated:

1. That the stain should be carefully examined in a strong light by a low power of the microscope. Its colour, consistency, and general appearance, may thus be noted.

2. That if possible a portion of the coloured substance should be removed and digested in a small quantity of water.

3. That another portion should be placed on a slide with water and glycerine, or a solution of iodide of potassium, and when a coloured liquid appears, this should be examined by a high power of the microscope for corpuscles or blood-cells. Their form, whether round or oval, should be noted, and their size determined by a micrometer.

4. That if the coloured substance resembling blood cannot be removed, the surface or substance of the cloth with the stain upon it should be cut out, separated into small fragments, and these digested in water in a watch-glass over which another watch glass is placed, or in a small tube.

5. That if sufficient liquid of a reddish colour is obtained, as a result of this contact with water, it should be placed in a deep narrow cell and examined by a spectroscopic eyepiece with a low power of the microscope. If two dark absorption-bands appear, one in the middle of the green rays and one at their junction with the yellow, this will show that it is the blood of some red-blooded animal.

6. In reference to chemical tests—1. One portion of the coloured liquid should be heated in order to observe whether the red colour is destroyed by heat, and whether a filmy opacity or brownish coagulum is produced. This destruction of colour by heat is a chemical property of the red colouring principle of blood; 2. Place a portion on opal glass or white porcelain and add a drop of a weak solution of ammonia. The red colour of blood is not changed to a crimson or green tint, like other red colouring matters of fruits, roots, and flowers; 3. To another portion on white porcelain add a drop of alcoholic solution of guaiacum; a reddish-white precipitate of the resin is formed, and on adding to this a drop of peroxide of hydrogen or antozonized ether, a blue colour speedily appears, varying in intensity according to the amount of red colouring matter of blood dissolved. On another part of the

porcelain the guaiacum and peroxide may be mixed in like proportions in order to compare the results. This experiment should also be performed with a watery solution of the red colouring matter of blood.

7. If no solid coagulum can be obtained from the article under examination, or if the stain has been so washed as to diffuse the blood in a most diluted form over a large surface or through the fibres, then the only process available is to apply the guaiacum and peroxide directly to the stuff, not only where it is stained, but where it is not stained, and after a time press these portions while still wet on a surface of white blotting paper. The blue colour not visible on the dark stuff will become visible on the white paper. This, when proper precautions are taken, will indicate the presence of blood.

8. This process, like the spectroscopic examination of blood, enables the operator to say that the results, if affirmative, show that the red colouring matter of a red-blooded animal is present. It does not indicate whether the blood has been derived from the human body, or from any one of the four great classes of animals,—mammalia, birds, reptiles and fishes. Then, in reference to the body, it throws no light upon the questions, whether the blood is arterial or venous, derived from the male or the female, or from a fœtus, adult or aged person. Under all these varied conditions the chemical results of the guaiacum process are the same.

It will be seen from these remarks that the use of guaiacum adds another and valuable chemical test to those hitherto employed for the detection of blood. It enables a chemist to speak with reasonable certainty to the presence of blood when in very small quantities, and to trace it in those cases in which an attempt has been made to remove the marks by washing. On the other hand, when the results are negative, it enables him to say that a suspected stain was not caused by blood—a fact of considerable importance in some medico-legal inquiries.