Sourcebook in Forensic Serology, Immunology, and Biochemistry

Unit IX: Translations of Selected Contributions to the Original Literature of Medicolegal Examinations of Blood and Body Fluids

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Sourcebook in Forensic Serology, Immunology, and Biochemistry

Unit IX: Translations of Selected Contributions to the Original Literature of Medicolegal Examinations of Blood and Body Fluids

compiled and edited by

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FOREWORD

Translations of Selected Contributions to the original Literature of Medicolegal Examinations of Blood and Body Fluids is Unit IX of a larger work with the title, Sourcebook in Forensic Serology, Immunology and Biochemistry.

Unit IX contains 50 translated papers arranged in four sections. Section I, on Identification of Blood, consists of 16 papers beginning with the early work of Orfila, using chemical means to identify blood, and differentiating rust, blood stains and stains from lemon juice on iron in resolving the medical-legal matter of wounds. The microscope was found useful by Mandl, Robin and Salmon identifying the formed elements, red cells with and without a nucleus (human and certain species of animal blood) and differentiating menstrual blood from blood drawn from a vessel by noting the mixture of blood cells with epithelial cells and leukocytes. In 1853, Teichman described hemin crystals, a crystalline chloride of heme, which he obtained from human blood and blood of the dog, rabbit, steer and other animals. Hoppe's 1862 paper gives the early report on the behavior of hemoglobin in the spectrum of sunlight.

In 1912, Takayama reported and expanded on the medicolegal applications of hemochromogen crystals; Donogany described the production of hemochromogen crystals in 1893.

In 1862, van Deen described the guaiacum test for detection of blood which was replaced with the more sensitive benzidine test reported by the Adlers in 1904. The last paper in Section I is the 1937 publication of Specht on the chemiluminescence of hemin in detecting blood stains.

Section II, on Body Fluids has 10 papers, mainly dealing with the detection of semen by microscopy and stains, the crystal test of Florence and finally, Lundquist's acid phosphatase test reported in 1945, which is useful in aspermic individual stains.

Section III, Determination of Species of Origin has 16 papers dealing with the applications of immunology in forensic serology. Essentially, these were biological protein differentiation and eventually, forensic blood differentiation reported by Uhlenhuth, and Wassermann and Schütze, within several weeks time, by use of the precipitin test. Neisser and Sachs reported on the application of the complement-fixation test as control and a supplement to the precipitin test.

Section, IV, Blood Grouping—Medicolegal Applications has 8 papers. Notable are the papers of Leone Lattes in which he brought to the attention of the "forensic camp" the Landsteiner rule sitting in the literature at that time for 12 years. Namely, that human bloods can be divided into four groups based on cells with or without A and/or B antigen; and serum with or without anti-A and/or anti-B antibody. One can determine this relationship from Landsteiner's study, but Lattes further explains that the division was expressed in this form only in the works of v. Dungern and Hirszfeld. Lattes also noted that the percentages of the single type (A-B-O gene frequencies), quoted independently of one another, by v. Dungern and Hirszfeld in Heidelberg, and by Moss in Philadelphia, were just about identical. The figures showed that 40% of all bloods tested had no A or B antigens on the red cells, but did contain two agglutinins, alpha and beta in the serum. These bloods were called group O.

In early articles, Lattes used the A-B-O nomenclature of Landsteiner: $A\beta$, $B\alpha$, AB, and $O\alpha\beta$ but in the 1927 paper he used the Landsteiner and Jansky nomenclatures (classifications) together: Group II ($A\beta$), Group I ($O\alpha\beta$). This is noted because during the 1930's the Moss, Jansky and Landsteiner classifications were used which resulted in some confusion to blood group serologists, physicians and blood bank personnel in general. The situation was finally corrected when the National Research Council recommended sole use of the Landsteiner classification to the Armed Services at the

beginning of World War II which was also the beginning of large scale blood transfusion.

Siracusa reported (1923) on his absorption experiments with stains to identify the group by allowing the A and B antigens to absorb A and/or B agglutinin, respectively. He further confirmed the specificity of the absorption by eluting at 45°C and testing the eluted agglutinins with A and B red cells.

Franz Holzer was an internationally know forensic pathologist. His contributions are well cited in the last three papers of Section 4. Holzer was one of Landsteiner's five students. His passing, followed by that of Wiener, leaves only three remaining: Philip Levine, Merrill W. Chase, and J. L. Jacobs. In our lifetime, their contributions have illuminated the scientific literature.

Professor Gaensslen is to be commended for his scholarly achievement and contribution to the English literature in bringing together this extensive series of translations with direct bearing on forensic serology, immunology and biochemistry. The translators, specialists, involved in this project have done an outstanding job.

The late A. S. Wiener described P. B. Candela's efforts in identifying the blood groups of ancient bones (mummies) as a labor of love. Certainly the same can be said of the Sourcebook and translation series which Professor Gaensslen and associates have worked so diligently to bring to a successful completion. The translations and Sourcebook will provide additional reference material for operational crime laboratories, academic institutions and research libraries. This availability to the workers, teachers, and research oriented staffs in the fields of forensic serology, immunology and biochemistry provides a means to achieve new excellence in medicolegal studies.

> Frank R. Camp, Jr. Louisville, Kentucky September, 1978

PREFACE

In the course of preparing the Sourcebook in Forensic Serology, Immunology and Biochemistry, I became aware of set of translations of selected important papers in blood grouping and immunohematology. Selected and edited by Col. Frank R. Camp, Jr., Col. Frank R. Ellis and Col. Nicholas F. Conte at the Blood Bank Center of the U. S. Army Medical Research Laboratory, Fort Knox, KY, the collection contains 36 original papers and monographs by Landsteiner, Hirszfeld, Bernstein, Friedenreich, Schiff, Dahr and others covering the most important developments in blood grouping serology and immunohematology. In addition, two theses written in other languages were translated. One was an extensive study of weak subgroups of A by Arne Gammelgaard; the other an extensive study of secreted group substances in body fluids by Grethe Hartmann. A few papers originally written in English, but old and virtually inaccessible, were included as well. This compilation was of enormous help to me in preparing the Sourcebook, since it was necessary to consult most of the papers which were contained in it. I was greatly impressed by the set of translations, and realized that, but for the efforts of Camp, Ellis and Conte and their collaborators, much of this now classical literature would not be available to those of us whose abilities in foreign languages are less than noteworthy.

I thought that a somewhat similarly conceived set of translations might be of some value in connection with the Sourcebook. There are a number of important papers in the original literature of the medicolegal examination of blood and body fluids which were written in languages other than English. The history and development of the field interested me, and I thought that it might interest others as well. I consulted with a number of colleagues and friends, and received encouragement to carry forward with the translations set. In addition, I wrote to Col. Camp in Kentucky and asked for his opinion about my plan. He was most encouraging, enthusiastic and helpful, not only in his response to my initial inquiry, but throughout the course of the project. This Unit of the Sourcebook is the result of the translations project. It was my strong feeling from the outset that the continuity between the set of translations prepared at Fort Knox and this project could best be expressed by asking Col. Camp to write a Foreword to this set. I am most grateful to him for having very kindly agreed to do so.

The selection of papers for inclusion in this set was not always easy, and for better or for worse, I alone am responsible for the choices. I wanted to include some of the very earliest works on the subject, those of the renowned Orfila and his collaborators in early 19th Century France. I wanted to include those papers which may now be regarded as classical, in that they represented the first reports of methods or techniques, some of which have survived to the present time. Other papers were chosen because they represented the viewpoints about certain procedures in various places at various times in the history of the field. The translations set is divided into four sections, corresponding approximately to the divisions of the main Sourcebook: Identification of Blood; Identification of Body Fluids; Determination of Species of Origin; and Blood Grouping. In some cases, the papers discussed more than one of these subjects, and the decision concerning which section they should be placed in was somewhat arbitrary. Each section has a brief introduction, the purpose of which is to explain something about the basis for the selection of papers and the way in which the different works relate to one another. I have also included such information as I have been able to discover about the authors of the papers, some of whose names are synonymous with tests that are still used today. Where possible, I have included photographs.

All of the papers which appear in the translations set are cited in the main Sourcebook. The work is discussed in connection with other work on the same or

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similar subjects. I have not, therefore, gone into the nature of the work in very much detail in the short introductions to the sections of this Unit.

An effort was made in preparing the translations to keep the language fairly literal, as close as reasonably possible to what was written by the original author. At times, this practice results in English which is not in the best syntax, or is somewhat convoluted in construction. We nevertheless thought that there was merit in following this practice.

A few conventions have been followed in setting out the translations: The original pagination has been included as a page number, followed by a slash, at the right-hand margin. All page number references in the texts are to the original page numbers, and not to ours. In some cases, the formats, especially of the title pages, have been rearranged to reflect more modern style. In the older literature, first name(s) or initials were sometimes omitted. In cases where the first initials or names of the authors were known, we have added them. In papers where there were very many footnotes, or references appearing as footnotes, we have placed these at the end of the paper in a reference list. In some cases, the reference numbers do not correspond to the originals, because it was the convention in some older journals to number the footnotes from Number 1 on each new page. But all the numbers have been adjusted so that the correct reference matches the reference number. It was necessary in a few places to add comments or remarks which did not appear in the original article. This we have done in square brackets. Photographs and drawings have not been included in the translations. The references in the papers have been left in their original form for the most part. They have not been edited, and may not all be accurate.

It is a pleasure to acknowledge the interest and assistance of a number of people who have helped to see to it that this project was completed. I want to thank the PSC-BHE Research Award Program and the Research Foundation of the City University of New York as well as the National Institute of Law Enforcement and Criminal Justice for financial support. Without this support, the work could not have been done.

All the translators did very excellent work, which is very much appreciated: Hannah DeVegh, Timothy Miller, Hugo Twaddle, Emile Capriotti, Rebecca Guenther and Kaori Kato.

A number of people were helpful with difficult passages in foreign languages, and with bibliographic material, including John Broadwyn and Dr. Stephen Kim at the National Library of Medicine.

I am very grateful to Prof. Dr. Hiroshi Hirose of Kyushu University, Fukuoka, Japan, for supplying me with a great deal of information about Dr. Takayama, for sending me the photograph of Dr. Takayama, and for assistance in obtaining permission to reprint the translation of the pyridine hemochromogen paper. Dr. George Sensabaugh of the University of California, Berkeley, was not only generally supportive, but helped to arrange for one of the translations.

A number of people were helpful in obtaining the photographs which appear in this unit, as well as permission to reprint them. I am indebted to Lucinda Keister, Curator of the Portrait Collection at the National Library of Medicine, for a number of the photographs. Prof. Dr. Hiroshi Hirose sent me the photograph of Takayama, and was extremely cooperative in many ways. I thank Prof. Antonio Fornari and Prof. Sergio Perugini in Pavia for obtaining the photograph of Prof. Leone Lattes, which originally appeared in *Haematologica*, volume 38, number 9 for 1954, and I am grateful to the editors of *Haematologica* for permission to use the picture. I want to thank Prof. Dr. Angelo Fiori in Rome for sending the photograph of Prof. Siracusa, and for obtaining the latter's permission to publish it. Verlag Franz Deuticke kindly extended permission to reprint a photograph of Prof. F. J. Holzer which originally appeared in *Beiträge zur Gerichtlichen Medizin*.

The kind permission of many publishers of the original articles which were protected by the copyright laws and agreements for the reprinting of the translations is gratefully acknowledged: Nordisk Medicin, Stockholm; Walter de Gruyter & Co.

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Verlag, Berlin; J. F. Bergmann Verlag, Munich; Verlag Schmidt-Römhild, Lübeck; Georg Thieme Verlag, Stuttgart; Springer Verlag, Heidelberg and New York; Edizioni Minerva Medica, Torino; Masson, S. A. in Paris; Verlag Franz Deuticke, Vienna; Verlagsgesellschaft Otto Spatz, Munich. A special word of thanks to Fiametta Lattes Treves of Milano, Italy, for giving permission on behalf of the family to reprint the papers of Prof. Dr. Leone Lattes. In this connection, I would also like to thank Prof. Dr. H. Jahrmärker, Prof. Dr. Friedrich Geerds, Prof. Dr. J. Gerchow, Prof. Dr. A. Fornari and Prof. Dr. Diethard Gemsa for handling various correspondence, and for their help in obtaining the permissions. I am grateful to Mr. V. Borsodi and Mr. W. Bergstedt of Springer Verlag for help in straightening out several problems with the permissions.

Maureen Swift typed the entire manuscript, accurately and efficiently, often from very difficult drafts, without once losing her good humor. Without her assistance, the work could surely not have been completed.

Washington, D.C. June, 1978

Col. Frank R. Camp, Jr. (USA, Ret.) died in 1983 before the final publication of this book. The earlier, similar work of Col. Camp and his colleagues served as a motivating factor in the undertaking of this project, and his assistance with and enthusiasm for it were important in bringing it to completion. Accordingly, this work is dedicated to the memory of Col. Camp and to his many contributions to immunohematology and serology.

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Prof. Mattieu Joseph Bonaventure Orfila 1787-1853 Courtesy National Library of Medicine

Prof. Ludwik Karol Teichmann 1823-1895 Courtesy National Library of Medicine



Prof. Dr. Masao Takayama 1872-1943 Courtesy National Library of Medicine

Section 1. Identification of Blood

The application of chemical methods to the identification of blood for medico-legal purposes has its essential beginnings in the studies of Mathieu-Joseph-Bonaventure Orfila. At the time the studies were published, he was one of the most highly regarded medical scientists in France, and perhaps in the world. He was born in Spain in 1787, and began his education there, but in 1807 he went to Paris where he stayed. He was primarily a toxicologist, and his Traité de Toxicologie Général went through five editions. A Traité de Médecine Légale went through four editions, and a Traité de Chemie went through eight. In 1811, Orfila received his doctorate at Paris, his studies and his career apparently having been helped considerably by the interest taken in him by the well known chemist L. N. Vauquelin (1763-1829). He became a physician to the Court of Louis XVIII in 1816, Professor of the Faculty of Medicine in 1819, and a member of the Academy of Medicine in 1820. In 1823, he was made Professor of Chemistry, and in 1830, Dean of the Medical Faculty at Paris. Over the course of his career, he became embroiled in a number of disputes over scientific issues, and it appears that he generally prevailed even when he was wrong. Reading through the papers in this section, it will be clear that F. Raspail disagreed with Orfila about the first blood tests. The two apparently had a number of disagreements over toxicological questions as well. Orfila's rejection of the microscope as a major tool for blood examination and identification and for seminal stain identification (see in Section 2) laid the matter to rest for a number of years. Orfila died in 1853, and an obituary appeared in the Annales d'Hygiène Publique et de Médecine Légale, of which he was a founder, in that year.

The case reported by Orfila, Barruel and Chevallier in 1835 is interesting in that the blood indentification procedure developed by Orfila, and the "odor test" for human species identification developed by Barruel (see in Section 3), were applied to case materials. The 1845 paper by Orfila is an assessment of a technique developed by Prof. Persoz at Strasbourg employing hypochlorous acid.

There was a controversy in the early 19th Century about whether microscopical examination or chemical tests should have priority in examining bloodstains, especially in regard to which of these should be regarded as giving more certain results. The same arguments existed in connection with seminal stain examination (Section 2). There was a further dimension to the arguments about the examination of bloodstains. Prévost and Dumas had found in 1821 that the red cells of different species differed in size, and this in addition to the fact that mammalian red cells are anucleate, while those of birds, repiles, etc. have nuclei. Many of those who favored microscopical examination of bloodstains

thought that these characteristics could be exploited for species differentiation. The finding of elements of blood (such as red cells) by microscopical examination was taken as incontrovertible evidence of the presence of blood. In Mandl's paper, a strong argument is made for the use of the microscope, and the early history of the subject is discussed. The papers of Robin and Salmon (1857) and of Robin (1858) strongly reinforce the recommendation of the use of microscopy for the assessment of blood and body fluid stains. Charles Robin (1821-1885) was a recognized histologist, and it is to the case report by him and Salmon in 1857 that Masson (1885) attributed the beginning of a new period in the history of medico-legal stain examinations, characterized by the use of the microscope as a primary tool (see in Section 3). A number of chemical tests are discussed by Rose (1853) in Germany. Indications are that he was familiar with the earlier French literature, but he makes no mention of the use of the microscope.

The crystal tests for blood have been considered by a number of workers to be certain proof of the presence of blood. The hematin crystal test grew out of the studies of Prof. Ludwik Teichmann (1823-1895) in 1853. Teichmann's biography may be found in the Bulletin of the Polish Medical Society 8 (3) for 1965. A tribute to him, which also discusses his life and work, appeared in Gazeta Lekarska 6 (26) for 1886. The other important type of blood crystal that has been used as a basis for medico-legal identification of blood is hemochromogen. These were prepared using pyridine in 1893 by Donogany. The classic paper on the preparation of pyridine hemochromogen crystals as a means of identifying blood for medico-legal purposes is that of Dr. Masao Takayama (1872-1943). This paper does not seem to have been correctly cited anywhere in the European or American literature since its publication in 1912. Takayama went to study with Prof. Kobert in Germany from 1904-1907, and it seems reasonable to suppose that his interest in hemochromogen crystals was kindled in those years. The laboratory was very active in these studies around this time. Dilling's Atlas in 1910 (see in Unit II, section 3 of Sourcebook) was the result of work in Kobert's laboratory at Rostock. Takayama spent much of his professional career in the Department of Legal Medicine at Kyushu University in Fukuoka, which he founded. He held important Directorships and Deanships of the medical faculties at Kurume and Nagasaki as well. For a time in 1936, he was Rector of Kyushu University. He is recognized in Japan for work he did on fingerprint images as well as for his work on blood identification and hemochromogen crystals.

The spectrum of hemoglobin and of its derivatives has been very important in the development of hemoglobin anal-

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ysis. Spectral tests have been used in the examination of blood stains as well. Hoppe (1862) first reported on the visible spectrum of hemoglobin.

The catalytic tests, which are almost universally employed at present for blood identification, are based on the peroxidase activity of hemoglobin and its derivatives. The procedure was introduced by van Deen in 1862, using guaiacum as substrate. The paper was signed "J. van Deen," but a Dutch biographical source lists him as "Izaak van Deen." He lived from 1805 to 1869. The guaiacum test was widely used until 1904, when Rudolf and Oskar Adler published their now classical paper on catalytic reactions using benzidine, the leuco base of malachite green, toluidine and tolidine as substrates. A variety of other oxidizable organic substrates were tested as well. The luminol test, which is not quite a catalytic test in the same sense as the others, has enjoyed some popularity, and is still used sometimes. This method was orginated by Specht in 1937. Specht lived from 1907 to 1977. During his career, he was in Jena, Halle, and later in Munich. He was associated with the editorial board of the *Archiv für Kriminologie*, and a brief tribute to him may be found in volume 159 of this journal, numbers 3-4 for 1977.

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Blood Considered in the Context of Legal Medicine Memoir Read at the Royal Academy of Medicine*

M. J. B. Orfila

65 Before delving into the section which is to be the particular object of this memoir, I feel it necessary to present succinctly the series of works which I propose to communicate in it. I will present, in a first memoir, the means necessary for differentiating rust, blood stains and stains from lemon juice on iron: this subject, as can be seen, is immediately connected to the medico-legal matter of wounds. In a second work, I will present the characteristics of semen stains, compared to those of fat and the matter of various discharges from the vagina and of the urethral canal, in acute and chronic leukorrhea, in gonorrhea, in chronic urethritis, etc. I will prove that it is not difficult to recognize if linen has been stained by semen and this will contribute much to the clearing up of certain questions relative to defloration and rape. The third memoir will treat asphyxia by submersion: numerous experiments done on living animals, and about fifty autopsies on drowned corpses, several of which remained in the water for several months, will enable me to carefully trace the medico-legal history of submersion and lead me to the remarkable conclusion that, except in very rare cases where slime, mud, gravel, etc., is encountered in the last bronchial subdivisions, and where the cadaver has

not been found in a vertical position, head on top, it is 66 impossible to determine if anyone has been submerged alive, unless wounds necessarily immediately mortal are discovered on the corpse, which indicate that the individual could not have thrown himself into the water. The meaning to be drawn from the presence or absence in the airways of an aqueous and sanguinolent froth will be of much less importance than was believed up to now. In the fourth memoir I will be concerned with poisoning from a new point of view. How do venomous substances, mineral and vegetable, behave in rotting animal matter? Up to what time can their presence or that of new compositions they have formed be demonstrated, supposing they have been altered? This question, of paramount concern to physicians and pharmacists charged with certain legal exhumations, will be resolved by experiments in which the principal mineral and vegetable poisons were mixed with animal matter that was left to rot for twenty months, either in containers exposed to air, containing water, or buried in earth: it is unnecessary to mention that, especially in the first case, the putrefaction was at its peak, and that the necessary elements to give a satisfying solution to the problem were consequently present.

[Section consisting of further discussion on the matter of poisoning and poison detection not translated.]

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Blood Considered in the Medico-Legal Context

Physicians are often required by courts to determine if 3t stains, noted on instruments of iron or steel or on linen, are produced by blood. This matter having been the object of a work published by Lassaigne in 1825, we feel it necessary to announce, in order not to be accused of plagiarism, that we have treated this subject since 1823 in one of our lessons at the Faculty of Medicine. Moreover, if our experiments have some resemblance to those of the distinguished chemist, it will be seen they differ in several respects, and especially, that they consider the question in a much more comprehensive manner. We feel it necessary to examine successively blades of iron or steel and stained fabric.

Blades of Iron or Steel

Stains produced by blood on these instruments can be confused with those produced by lemon juice and rust. It is of consequence, then, to study them comparatively.

Characteristics of Dried Blood Stains

Points of the blade on which there was only a small quantity of blood were light red; they presented, by contrast, a dark brown color where blood was deposited in greater quantity. If the portions of this blade, where a layer of blood of appreciable thickness is found are exposed to a temperature of 25° to 30°, they drop off in scales and leave the metal rather shiny. In heating a portion of dried blood in a small glass tube, a volatile ammonia product is obtained which turns the color of litmus paper blue; the paper was arranged on the upper part of the tube prior to heating. When a drop of pure hydrochloric acid is placed on the dried blood stain, the stain doesn't yellow or disappear, nor does the iron become shiny, as happens with a stain produced by lemon juice or rust. In immersing the stained portion of the blade in distilled water, reddish striations quickly appear, running from top to bottom, and the coloring substance is soon found gathered at the bottom of the liquid which remains uncolored with the exception of its lower portion. If, at this time, the blade is withdrawn, the stained parts, thus treated with water, show whitish or slightly reddish white filaments.

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^{*} Translation of: "Du Sang, considéré sous le rapport de la médecine légale; Memoire lu à l'Académie royale de Médecine".

in Journal de Chimie Medicale de Pharmacie et de Toxicologie 3 (8): 365-374 (1827).

These filaments, formed by the fibrin of blood, could very well not be perceptible if the stain being operated on is of small thickness. The aqueous liquid from which the iron

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blade was withdrawn was shaken in a glass tube, and acquired a rose or red color, according to whether it involved a greater or less amount of coloring substance. It enjoyed remarkable properties; it doesn't reestablish the color of litmus paper reddened by acid; chlorine used in small amount colors it green without precipitation. If more is added, it discolors the solution without making it lose its transparency, but soon after, it colors the solution opaline, and finishes by forming a deposit of white flakes. Ammonia doesn't visibly change the color, whereas it alters several red vegetable colors, like cochineal, Brazil wood, etc.; nitric acid gives rise to a greyish-white precipitate and the solution is almost discolored. Concentrated sulfuric acid gives rise to a similar precipitate only when used in rather large quantity; potassium ferrocyanide doesn't trouble it in any way; aqueous solution of gall nut provokes a precipitate of the same nuance as that of the liquid. The liquid discolors after filtration, or at least only conserves the yellowish color of dilute gall nut. Submitted to heat, the liquid of which we are speaking coagulates, unless it is very diluted in water, where it simply becomes opaline at first and only coagulates when a notable quantity of water is evaporated by boiling.

If, instead of withdrawing the iron blade stained with blood at the moment when the liquid is colored red in its lower part, it is left for several hours in water, in contact with air, the iron passes into the state of reddish-yellow trioxide, which remains in great part suspended in solution and imparts a yellowish tint to it. Another part of the trioxide, in

/370 depositing, mixes with the red coloring substance occupying the bottom of the container and alters the color; but filtration suffices to separate all the trioxide, and the solution then passes to clear, colored in *light rose, deep red*, or *red* and shares all the properties which we have just assigned to water colored by blood. If the water in which the stained instrument was immersed contains only a small amount of coloring substance, or, in other terms, if the stain was not very sensitive, the solution will be troubled once again by gall nut or nitric acid.

Characteristics of the Stain Formed by Lemon Juice (Iron citrate)

When lemon juice is deposited on an iron blade exposed to the air, iron citrate of a reddish-brown, which is possible at first glance to confuse with dried blood, quickly forms. A man was recently suspected of having murdered another. Found on his chimney was a knife which appeared blood stained. This new burden seemed to overcome the accused; then it was learned at the laboratory of the Faculty that these presumed blood stains were only iron citrate produced by the simultaneous action of air and citric acid on a knife that wasn't wiped when, several days beforehand, it was used to cut a lemon. The points of the iron blade on which there

was only a small amount of lemon juice were a yellowish red, whereas they present a dark brown color similar to that of dried blood when the juice was used in a stronger proportion. In the latter case, the stain scales, the iron citrate detaches and leaves the metal shiny when the temperature rises to 25° or 30°. If a portion of this citrate is heated in a small glass tube, a volatile acid product is obtained; also, litmus paper placed at the upper part of the tube, and moistened beforehand, quickly turns red. In placing a drop of pure hydrochloric acid on the stain we are discussing, the liquid yellows and the iron becomes shiny in the same instant; iron hydrochlorate is formed. Also, distilled water with which the stain is washed, after treatment with hydrochloric acid, furnishes a precipitate with potassium ferrocyanide and gall nut similar to that produced with a saline solution of iron. In immersing the stained portion of the blade in distilled water, the iron citrate quickly dissolves and colors the liquid yellow. This solution reddens litmus paper, gives a more or less dark violet precipitate with gall nut, a green or red one with alkali, according to whether the iron is in the state of dioxide or trioxide, and a blue one with potassium ferrocyanide. Sometimes, to attain this last hue, it is necessary to add a little chlorine.

Characteristics of Rust Stain (Iron trioxide subcarbonate)

The color of this stain is yellowish-red, ochre yellow, or red. Exposed to a temperature of 25° to 30°, the blade thus rusted does not scale, as happened with stains of blood and lemon. Heated in a glass tube, rust gives off ammonia, as was demonstrated by Vauquelin and Chevallier; also, reddened litmus paper, placed on the upper part of the tube in which the experiment was being done, turned blue. A drop of pure hydrochloric acid placed on the rust turned yellow in an instant; the stain unrusted, and in diluting the acid with distilled water, a yellowish solution behaving like iron salts toward reagents is obtained. Put in distilled water, the rust is not dissolved at all; however, it detaches and remains suspended in part in the water, in part at the bottom of the container; the solution yellows because of the rust in suspension, but it suffices to filter it to decolorize it, which never happens with an iron blade stained with blood or iron citrate. This filtered solution, holding no iron in solution, when examined a few hours after the beginning of the experiment, does not become clouded by alkali, gall nut, or potassium ferrocyanide.

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Fabric Stained by Blood

If the layer of blood is of a certain thickness, and the stain is formed by all the materials of blood with the exception of water, a portion of the fabric stained in red brown is cut out and immersed in distilled water. The coloring substance of blood detaches soon after, traverses the liquid from top to bottom in the form of red striations, and gathers at the bottom of the container, whereas the supernatant water is scarcely colored. At the end of a few hours, when the col-

oring sustance is dissolved, at least the greater parts of it, in place of the stain will be found fibrin of blood, in the form of soft matter of a greyish or a rose white which is easily removed by a finger nail. The more this layer was whitened by the water and the browner the fabric on which the blood was applied, the more apparent will the layer be at first glance. In the case where it is of a hue too dark to be recognized, the cloth is immersed once again in pure distilled water for a few hours, to remove another portion of coloring matter. The solution, at the bottom of which is found gathered this material, is shaken in a glass tube, and shows a reddish color, behaving with heat, acids, chlorine and other reagents as we have already described in our presentation of an iron blade stained with blood.

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If the stain, instead of presenting a notable thickness, is the result of simple absorption by the material, as happens when parts of the linen surrounding the portions on which the blood had been applied, are examined, or if it comes from other blood stains which were scrubbed or washed after drying, it will be impossible to confirm the presence of fibrin, for this does not exist in stains resulting from absorption and would have been detached in the case of rubbing or washing. One is limited then to separating the coloring matter by distilled water. The solution is subjected to the same procedures as in the preceding case, and if it has the characteristics already presented, the stain can be affirmed as being formed by the coloring matter of blood, provided that none of the substances with the property of coloring the water red or rose (cochineal, Brazil wood, cartham, madder-wort) furnish a liquid behaving with heat, and all the aforementioned reagents, like aqueous solution of blood.

The preceding experiments were done in turn with human blood and with the blood of beef, sheep, dog and pigeon.

It will not be useless, in ending this work, to note successively the manner in which the major red coloring substances

behave with the reagents which we said must be used to identify the coloring principle of blood.

Cochineal. A solution of dilute cochineal is the color of red currant. Ammonia changes it to violet without clouding it. Solution of gall nut doesn't give a precipitate. Sulfuric acid and nitric acid, far from giving a precipitate, render it more 374 transparent and give it a scarlet color. Potassium ferrocyanide does not cloud it, but darkens the color a bit. Chlorine completely discolors it without turning it green or giving a precipitate. If the solution of cochineal were concentrated, chlorine would yellow it and after a certain time produce an abundant flocculent, yellowish deposit.

Brazilwood. Diluted with water, the solution of Brazilwood is an orange red. Ammonia renders it violet without clouding it; gall nut gives no precipitate. Sulfuric and nitric acids change it to a fallow yellow, without making it lose its transparency. Potassium ferrocyanide darkens the color a bit. Chlorine does not cloud it, and changes it to yellow without a change to green.

Red substance of madder-wort dissolved in alcohol. When diluted with water, its color is analogous to that of the coloring substance of blood. Ammonia deepens the color. Solution of gall nut does not trouble it. Sulfuric and nitric acids yellow it and render it cloudy. Chlorine yellows it first, then changes it to green, and finishes by discoloring it without the solution even becoming opaline. It is seen by these experiments that if this substance resembles the coloring principle of blood in some aspects, it nevertheless differs enough so that they cannot be confused.

Red substance of cartham. It is yellowish when diluted in water. Ammonia deepens the color. Solution of gall nut gives a yellow precipitate. Sulfuric and nitric acid cloud it without changing its color. Chlorine discolors it right away without rendering it opaline.

Blood Considered from the Medico-Legal Viewpoint*

M. J. B. Orfila

1413 It is noted in the Journal de Chimie Médicale of last August "that on the occasion of a memoire of Orfila, Monsieur Dulong observed that one of the most marked characteristics of blood stains, even if very old, is the form of blood cells seen by the microscope; it permits, in addition, differentiation of blood of different classes of animals: dried mammalian blood cells look like a white disc surrounded by a red circle, whereas in the blood of birds the white disc is surrounded by an elliptic globule. This mode of examination is that much more valuable since it requires minimum quantities for its use and it doesn't deprive the substance of any material used in the application of analytical procedures". [Compte rendu de la seance de la Societe philomatique du 14 July¹].

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Such a positive assertion, uttered by a scientist of this caliber, must leave no doubt as to the possibility of recognizing in every case, and with facility, not only if a stain is formed by blood, but again, in certain circumstances, to which class of animals the blood producing it belongs. We don't think that the problem is as easy to resolve by microscope, based on the facts we have observed with the greatest care, and which have been verified by Lebaillif, whose ability and experience no one will contest in any area of microscopical investigation.

1. Dried human blood on a glass slide. This blood came from a fingerprint; it had been diluted with about an equal weight of water and placed on the glass slide, where it dried out eight years ago. A very large number of perfectly *spherical* cells, transparent in the center, is seen; many of them were grouped together, forming an aggregate, which didn't hinder distinguishing them clearly. In examining a drop of the same blood, but thicker, with the same microscope it was impossible to perceive any distinct blood cells.

2. Human blood dried on cloth. Human blood was deposited on a piece of cloth as it poured from the vein. Four months later, a strip of this cloth, stained by a considerable quantity of blood, was left in water for an hour, until the liquid was colored red. Two drops of this solution were placed on a glass slide, at a certain distance from each other, and were examined after complete dessication.

The first drop, thick and wide. Perfectly spherical blood

cells, transparent in their center, can be seen at many points. In another area, there are, in addition, those whose form is not easy to determine; finally, elsewhere are perceived those which are round and others which are *elliptical*.

The second drop, small, not very wide. It is impossible to find any blood cells in the center of this drop; irregular bodies from the cloth on which blood deposited can be seen. In another part near the center two transparent, rather voluminous spherical blood cells can be seen, along with many others equally transparent and much smaller, one form *less regular*, difficult to determine, and others which are somewhat elongated.

3. Human blood dried on cloth, diluted in water, and viewed in the microscope before dessication. A portion of the blood which had been used in the preceding experiment was placed on a glass slide after dilution in water and examined while still liquid. A very large number of small, *transparent*, ovoid and spherical corpuscles were seen. In another part, it was difficult to recognize perfectly spherical corpuscles. A portion of the same drop which had been deposited on the glass slide, such that there was but a slight layer of blood, showed transparent, *elliptical* corpuscles; others spherical, but smaller in number; others were of an irregular form, and several were elongated, opaque bodies, undoubtedly coming from wool, dust and other refuse soiling the fabric.

4. Pigeon blood dried for six days on cotton linen. A portion of this linen containing all the substances of blood was left in a small amount of water until it was sufficiently colored. Three drops of this solution were desposited on a glass slide and were examined after complete dessication.

First drop, rather thick. In the center are some small, transparent, *irregular* bodies; between the center and periphery there are *elliptical*, square, spherical and triangular bodies and, in addition, there are opaque particles of black, which are square, triangular, etc; at the periphery, the mass is cracked from the dessication and colored in a more intense red and no transparent blood cells can be seen.

Second drop, much less thick. Only some opaque, elliptical, square and spherical corpuscles, which are not red blood cells, are perceived in the center. At the periphery can be seen a perfectly *spherical*, transparent blood cell, another elliptical, and several *opaque* corpuscles of different forms.

Third drop, very thick. The center presents several transparent corpuscles, of irregular form, and others triangular, trapezoidal, etc; there are also opaque particles of varied forms. In a point near the central part there is an agglomeration of transparent corpuscles, whose form is not easily identifiable. Finally, there are neither blood cells nor cor-

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^{*} Translation of: "Sur le sang, considéré sous le point de vue médico-légal". in Journal de chimie médicale de pharmacie et de toxicologie 3 (9): 413-419 (1827).

¹ In this same meeting Adophe Brongniart noted that blood of beef had been differentiated from human blood by microscope by Monsieur Dumas in a medico-legal case... Brongniart probably confused this fact with another, since Dumas said he knew nothing about it.

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puscles near the periphery, where the mass is cracked and deeply colored red.

5. Pigeon blood dried on cloth, diluted in water, and seen under the microscope before dessication. A portion of the blood which had been used in the preceding experiment was deposited on a glass slide after being diluted with water and examined while still liquid. A piece of elongated, opaque cotton is seen to which a multitude of transparent corpuscles of different forms seem to adhere. At another point in the drop, blood cells are seen in rather a great number, *elliptical* for the most part and isolated; others which appear to have the same form are agglomerated. Elsewhere can be distinguished two *perfectly spherical*, transparent blood cells, *comparable in every way to those of human blood*, and beside them are three more which are elliptical.

6. Pigeon blood dried on cotton cloth for six days and 7 containing only the coloring matter. A portion of this cloth, adjacent to the area where the blood had been deposited, and containing no solid parts, was treated with water, as previously. When the solution was sufficiently colored, it was placed on a glass slide and examined after dessication.

First drop. In its center are seen a considerable quantity of dendrites and a few rounded and fringed corpuscles. The periphery presents no foreign bodies and a yellowish-red hue. Second drop. With regard to the periphery, there is absolutely no difference between the two drops; as for the center many dendritic crystals and no rounded corpuscles at all. In the third drop numerous ramifications analogous to the preceding and some fringed, irregular corpuscles, apparently elements of crystallization, are seen; along with these are other corpuscles, more rounded, but so tenuous that the form can't be exactly determined; and other corpuscles, of a spheroidal form, very transparent at their center, which have a smaller diameter than human blood cells, of which we had first been speaking, but of just about the same form.

It results from the preceding, and from other facts which we will pass over without reference, 1) that, granting that the blood contains a multitude of blood cells serving to characterize it, it is sometimes impossible to determine the pres-

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ence of these blood cells in blood dried on a glass slide, and even more so on fabric, either because the drop of blood is too thick, or because it contains only the coloring matter (cf. section 6 above), or any other reason; 2) that though it is true, in general, that mammalian blood cells are circular, while those of birds and cold-blooded* animals are elliptical, it is no less certain that in a matter of blood *detached from cloth, elliptical* blood cells can be perceived in mammalian blood, and spherical blood cells, as well as triangular, square, etc. corpuscles in the blood of *birds*, probably resulting from an atom of dust or of the material of the fabric which unites with the blood. It is easy to imagine that a blood cell which had been spherical when seen alone, presents another form when coupled to a foreign corpuscle.

Let us add that it appears, from numerous observations done by Hewson, that in animals in which are found the very representative elliptical corpuscles after a certain time of life, only circular blood cells are found when they are very young (*Hewsoni opera omnia, Tabula prima, Lugduni Batavorium,* an 1785). And isn't it well known, moreover, how difficult it is to do good microscopical observations when not accustomed to them? These diverse considerations lead us to attach less importance to these observations than was believed necessary for resolution of the problems engaging our attention, and to prefer the chemical characteristics we discussed in our memoir on blood.

To justify this conclusion, we feel it necessary to point out that after examination of human blood and pigeon blood detached from fabrics, during several sessions and with several repetitions with excellent microscopes, not only was it difficult to distinguish one from the other, but even sometimes to recognize that it was blood. Let us now consider the quandary in which a physician, who hasn't devoted himself to microscopical research, would find himself. It might be said that we started off wrong, that we haven't fulfilled all the conditions. Very well! But we then request, in our turn, that these diverse conditions, and especially the numerous sources of error which can be committed, be indicated.

^{*} poikilothermic.

New Memoir on Blood Considered in a Medico-legal Context^{*†}

M. J. B. Orfila

- /105 At the meeting of the 15 of this month, the section of medicine heard the reading of a memoir of Raspail, having as its objective to prove that neither the microscope nor chemical experiments can identify blood stains. This memoir was addressed to the Royal Academy of Medicine by the author, as he claims in the covering letter, only because six months before I spoke to this association on the same subject and concluded, on the contrary, that it was possible to confirm the presence of these stains. Raspail, aware of the importance of the question he was treating, thought it not appropriate to leave any longer in error the numerous French and foreign physicians and pharmacists, who could have accepted my work as a guide. Nor did he economize any means for attaining the proposed goal: not only did he create out of whole cloth blood, which according to him shared all the characteristics I had allotted to human blood, but he also gave the prospect of the possibility of discovering someday at least twenty substances enjoying the same properties. He went further; he claimed that it was only up to a certain point, and not in an absolute way, as I have said, that human blood can be distinguished from iron citrate and iron trioxide, madder-wort, Brazil wood, cocchineal, earthame.
- 7106 To listen to Raspail, I would have to esteem myself happy if science conserved a slight memory of my work. The section easily felt that it is scarcely permitted me to keep silent in those circumstances; and more so since I am certain that, since the publication of my memoir, it has been concluded more than once before the courts of the kingdom that certain stains have been formed by blood because they presented the characteristics I had indicated. I will approach this question frankly.

The memoir of Raspail is composed of two distinct parts, one with chemical experiments as their objective, and the other, observations by microscope. I will say first, relative to the latter, that I won't bother with it, since it is in no way contradictory to what I had advanced. I will only recall to Raspail, and to the section, that I read on last August 21, in this context, a note in which I *already* established the insufficiency of the microscope in identification of bloodstains on fabric. (See the *Journal de Chimie Médicale*, issue of September, 1827).

Raspail's chemical experiments tend to establish: 1) the

existence of a red substance into which stains similar to those of blood can be made; 2) that no one can be assured that, one day, twenty substances capable of putting in error the reagents which I recommended for the identification of blood won't be discovered. Let's examine each of these points.

A. There exists a red substance with which stains similar to those of blood can be made. This substance is none other than eggwhite of hen left for a few hours in a canvas sack filled with powdered madder-wort, slightly moistened with water; the mixture is then exposed to a temperature of 25 to 30 degrees centigrade to dry it, and to give it the appearance of a red stain.

I don't doubt that Raspail borrowed from my first memoir on blood the idea for preparing such a substance: "When this substance is diluted with water its color is *analogous* to that of the *coloring substance of blood*. Ammonia deepens this color; a solution of gall-nut doesn't cloud the solution; sulfuric or nitric acid yellow it and render it a bit cloudy; chlorine yellows it first, then turns it green, and finishes by discoloring it, without it becoming even opaline. It is seen by these experiments that this substance resembles, *in some of its aspects*, the coloring principle of blood; it differs enough, however, so as not to be confused with it" (*Journal de Chimie Médicale*, issue of August, 1827).

But it is of little consequence to science that a discovery was realized here or further on; what is of concern is to find out whether the enunciation of a new fact is true or false. In this case, there is no doubt. Raspail is wrong.

Let someone repeat the experiment, as he recommends in his memoir, with a whole egg white and some madder-wort, without the addition of water, or after dilution of the eggwhite in three or four times its volume of water; it is left in contact with the madder-wort for a few hours. A substance is obtained which, dried at 25° or 30° centigrade, presents a red color, whose properties I am going to compare to dried blood.

Physical properties. To distinguish these two substances, one can, in the extreme, benefit from the differences in color and transparency existing between blood and the completely dried mixture of albumin and madder-wort. In effect, the hue of this last mixture will never be the same as that of blood, and it can happen, when too little madder-wort is used, that it is so different it becomes useless to turn to other characteristics. But I willingly acknowledge that these physical properties are insufficient for establishing this distinction when the artificial mixture is strongly colored; this will not hold for chemical characteristics.

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Translation of: "Nouveau Memoire Sur le Sang, considéré sous la rapport médico-légal".

in Journal de chimie médicale de pharmacie et de toxicologie **4** (3): 105-117 (1828).

[†] Read at the Royal Academy of Medicine January 29, 1828.

Chemical properties. In comparatively treating these two materials with cold distilled water, blood releases its coloring matter to the water and leaves fibrin in the form of more or less colored filaments, depending on how well they were washed; but in no case does this fibrin dissolve in the liquid. The mixture of dried eggwhite and madder-wort, on the contrary, treated in the same manner, releases both coloring matter and albumin to the water, considering that, after dessication at 25 or 30 degrees centigrade, this is very soluble in this cold liquid, a fact which certainly must have escaped Raspail. I will say, however, that if the egg white has not been diluted with water and was filtered before drying, cold water does not completely dissolve the red stain and that there remain some light filaments, which are only released corpuscles seen swimming in the liquid when egg white is shaken in three or four parts water. But it is impossible to be mistaken; the amount of undissolved substance is scarcely discernible, unlike a bloodstain treated with water. Moreover, it is easy to recognize the filaments of egg white.

The aqueous solution coming from the action of water on 9 the stains of the two substances presents the following differences:

1) It is orange-red when colored by madder-wort, whereas in the other case, it is brown-red.

2) Heated in a glass tube just to boiling, it coagulates or only becomes opaline, depending on the content of albumin; but if it comes from a mixture of madder-wort and egg white, it furnishes a rose-yellow or red liquid and a roseate coagulum, a part of whose color can be removed by washing with water. Whereas, blood gives a liquid and a coagulum of a greenish-grey without the lightest trace of a red hue. This greenish-grey coagulum can be rapidly dissolved with potassium and the liquid then acquires a red-brown color when seen by refraction. This important difference is known by every chemist and even by the worker dyers using madderwort; and I was astonished to see Raspail was unfamiliar with it. There's more: if the mixture of madder-wort and egg white were to lose its red color by boiling, as does blood, the operation bearing the name "madder-worting" would not exist. Indeed, in dye workshops, when dyeing cotton red, isn't 400 pints of water boiled with fifty pounds of madderwort and just about as much beef blood¹? If, in coagulating, albumin of blood caused madder-wort to lose its red color, there would be no possibility of tinting in this color. Besides, I can assert that, having boiled a mixture of beef blood and decoction of madder-wort, the solution conserves its red color, instead of the greenish-gray color which coagulated blood presents.

3) Nitric and sulfuric acids coagulate solution coming from blood; the clot is rose-grey and the supernatant, when left to deposit, is uncolored and a bit cloudy. The liquid mixture of albumin and madder-wort, treated by these acids,

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also coagulates, but the clot is straw-yellow and the supernatant is yellowish.

4) Solution of gall nut, made in the cold, coagulates the blood in rose-grey, whereas the alleged blood gives a yellowish-white precipitate.

5) Solutions of alum and stannic chloride only dilute the color of blood, *without changing it*. On the contrary, the mixture of albumin and madder-wort is yellowed by these solutions.

6) Concentrated alcohol gives rise to a *meat-red* coagulum at the end of a few hours, unless the solution of blood is too dilute. The filtered liquid is completely uncolored, whereas alcohol and the alleged blood give a *rose* coagulum, and a solution which, when filtered, is fallow bordering on rose.

7) Ammonia doesn't alter, or scarcely alters the color of blood, whereas that of a mixture of albumin and madder-wort changes appreciably toward violet.

8) Let us add that pure, concentrated hydrochloric acid does not yellow the bloodstain, but it browns the color more; the solid mixture of albumin and madder-wort, on the contrary, passes gradually to yellow by the action of hydrochloric acid, such that 20 to 25 minutes are sufficient for this hue to be very evident².

Will there be objection, by any chance, that the two solutions of which I've been speaking up to now present such clear cut differences only because they were not very dilute, and that the contrary would certainly be observed in examining very small stains? I would reply that in comparatively treating a bloodstain and a colored albumin stain with 16 grains of water, each stain weighing one-fifth of a gram, all the preceding indicated phenomena take place, and the characteristics are so clearcut that there is no doubt that a fifteenth of a grain of dried blood dissolved in 10 grains of water can be identified.

After all these facts, so positive, how can it be that Raspail claimed that a mixture of albumin and madder-wort cannot be distinguished from blood? How is it he was not aware that, in the interest of humanity, to use his expression, I would seek to give the greatest publicity to his so-called discovery and that, at this very time, facts incapable of sustaining the most superficial examination are perhaps being verified everywhere? Here, I believe, is the most plausible explanation of this inconceivable conduct of Raspail: he saw 112/ that heat, sulfuric and nitric acids, and solution of gall nut coagulate a mixture of albumin and madder-wort, and because these reagents also coagulate blood, he concluded they were identical!!! A strange way to reason: it would be necessary to conclude that iron and mercury salts are also

¹See the *Elements of the Art of Dyeing* by Berthollet, Vol. 2, page 158, 1791 edition; and *Elementary Course in Dyeing*, by Vitalis, page 324, 1827 edition.

² It is useless to recall that in medico-legal research relative to bloodstains, it must never be forgotten to treat the red solution with chloride and with potassium ferrocyanide, as I pointed out in my first Memoir. If I haven't discussed it here, it is because I wanted to mention only those reagents which can serve to distinguish blood from the substance prepared by Raspail and, in effect, chlorine and potassium ferrocyanide behave with this substance almost like they do with blood.

Identification of Blood

identical because both are precipitated by hydrosulfates, potassium, sodium, ammonia, potassium ferrocyanide, etc. Certainly not. And just as it suffices to examine the color of the different precipitates to distinguish iron salts from mercury salts, blood can likewise be distinguished from albumin, colored by madder-wort, for the four above-mentioned reagents give rise to coagula of different colors, and the supernatant liquids are also differently colored.

B. The first proposition advanced by Raspail having been completely refuted, I pass to the second, which is: No one can be assured that one day twenty substances will be discovered capable of placing in error the reagents used by Orfila to identify blood. "In organic chemistry, where almost all is in chaos, or almost all is mystery", said Raspail, "who would dare to assure me that twenty substances won't be encountered capable of placing my reagents in error by the versatility of their characters and the delicacy of their combinations?" (the Memoir cited). It is easy to see how difficult it is, when led into in the realm of possibilities, to assert nothing beforehand. I strongly doubt, however, that any such results will occur. And I sincerely urge Raspail, in the interest of legal medicine, to seek the compositions he foretells. When he has found them, I will agree with him: Blood cannot be identified by chemical means. In waiting, I affirm,

/113 the contrary.

I would be at fault if I didn't take this opportunity to point out the importance of the last question raised by Raspail. Legal medicine offers little else of such great interest.

[Section dealing with poisons and toxicology not translated].

I will end this memoir, already too long, with the following /115 conclusions:

1) In claiming that bloodstains on fabric cannot be identified by microscope, Raspail was in accord with the truth, as I had demonstrated before he did.

2) In denouncing chemical experiments as insufficient for identifying these same stains, and notably to distinguish them from stains produced by a mixture of albumin and /116 madder-wort, Raspail has committed one of the most grievous of errors.

3) In presenting this new proposition, that a substance cannot be confirmed as blood because several substances

resembling it might later be discovered. Raspail establishes a medico-legal principle it would be dangerous to adopt and which is even rejected by a healthy logic.

I recall to the section that at the last meeting, I urgently requested it to name a committee charged with reporting on the memoir of Raspail; I also asked to be admitted before this committee to prove to it the inaccuracy of the results proposed by the author of the memoir. I again demand a favor which the Academy will undoubtedly be eager to accord me: that Raspail be invited to assist at the work of the commission and that the experiments be done with materials prepared by himself.

I cannot urge too much that those physicians and pharmacists, most often called before the courts to judge questions of legal medicine, repeat the experiments of Raspail and compare his would-be blood with real blood. They cannot fail to recognize with me not only that there exist the differences between the two substances I have just pointed out in this work, but also that it is easy to distinguish them in considering only those characteristics I had already pointed out in the memoir read to the Academy in July, 1827. I discussed the action of water and of hydrochloric acid on solid blood and that of heat, sulfuric and nitric acids, ammonia and aqueous solution of gall nut on aqueous solution of blood. All one need do is to look to be convinced that these varied reagents act otherwise on blood than on the mixture of albumin and madder-wort. Moreover, it doesn't appear that our famous Vauquelin is disposed toward adopting the new ideas of Raspail; for, having been called upon, together with Barruel, on February 4 of this year, to determine if stains on a hat, smock, pants and shoe were produced by blood or not, he replied in the affirmative, as can be assured in reading the report he addressed to Sir Vanin de Courville, the examining magistrate. Vauquelin was acquainted with the experiments Raspail had read to the Society one month before. Even more remarkable, is precisely the fact that the conclusion of Vauquelin and Barruel was affirmative only because the material which they examined had the characteristics I had allotted to blood in my first memoir³. Will Raspail respond, by any chance, that the illustrious chemist which Europe has placed in the first order of analysts, and whom the courts have so often consulted, has not understood the question?!!!

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³ Vauquelin limited himself to confirming the physical properties of stains, to treat them with water, and to submitting the aqueous solution to heat, chlorine and gall nut. (See the report already cited).

Bloodstains. A Medico-legal Report*

M. J. B. Orfila, J.-P. Barruel and J. B. A. Chevallier

We, the undersigned, ... charged by the writ of Mr. Gaschon, examining magistrate of the court of first instance of the department of the Seine on the days of July 1st and 5th, 1834, as a consequence of the rogatory commission of June 22, 1834, executed by Mr. de Saisseval, examining magistrate of the Chateau-Thierry district in the affair conducted against men named Jean-Baptiste Boileau, Alexandre Boileau, Jean-Louis Boileau, and Victor Darez, all four accused of voluntary homicide committed in collusion, the 2nd of the said month of June, on the person of Mr. Hochet, a rural constable, with proceeding with the examination of garments and objects attached to this rogatory commission and with the operations necessary to respond to the following questions as much as possible:

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First question. Is it possible to determine if the blood mixed with earth, seized in the woods of Mesnil, is human blood, if it is from the same man as the blood found on the clothing of Hochet, on the clothing of Jean-Baptiste Boileau and on that of Victor Darez?

Second question. Is it possible to determine if the traces noted on the clothing of Jean-Baptiste Boileau, are traces of blood; if this blood, in the case of an affirmative finding, is blood of man or the blood of hare; if it is from the same man as that found on the clothing of Hochet and that mixed with earth from the woods of Mesnil; and, finally, if these blood stains have been on the clothing of Jean Baptiste Boileau for about three weeks or four months or even more?

Third question. Is it possible to determine if stains noted on the clothing of Darez are bloodstains and if this blood, in the case of the affirmative finding, is the blood of man or the blood of sheep; if it is from the same man as that found on the clothing of Hochet and as that found mixed with the earth of Mesnil and, finally, if these blood stains have been on the clothing of Victor Darez for three weeks or for five weeks?

Fourth question. Is it possible to determine if traces noted on a piece of blue cloth, found near the place where the earth of the woods of Mesnil was taken are blood stains; and if in the affirmative, if this blood is from the same man as that existing on the clothing of Hochet, on that of Jean-Baptiste Boileau, on that of Victor Darez and in the earth from the woods of Mesnil?

To conform to the requirements of the writ, the experts met at the chemical laboratory of the Faculty of Medicine, Monday the 7th of July at nine o'clock in the morning to be

sworn by Lafontaine, police commissioner attached to the office of judicial delegations, and to faithfully fulfill the mission confided to them.

The oath taken, the objects designated by the rogatory commission of Mr. de Saisseval were turned over to them, these consisting of:

- 1) a sandstone pitcher containing earth, leaves, pebbles and moss taken from the woods of Mesnil;
- 2) the bloodied garments of Hochet;
- 3) a piece of blue cloth;
- 4) a sorry-looking smock, blue on both sides;
- 5) trousers of blue cloth, patched and torn in several places;
- 6) a pair of old, large clogs;
- 7) a shirt of coarse white cloth;
- 8) a smock of old blue cloth;
- 9) another smock of newer blue cloth.

The experts having determined that all these objects, contained in large white wooden box bearing the address of the Crown's prosecutor, were furnished with appropriate tickets attached to the objects, took custody of them and adjourned until the following day, July 8, to proceed with the necessary operations and to reply to the questions posed by the rogatory commission delivered by Mr. de Saisseval the 22nd of June, 1834. On the said day of July 8, 1834, the experts met 352 once again in the laboratory of the medical school, where they proceeded in the following manner:

Examination of the clothing of constable Hochet. This clothing was in a packet formed by a piece of home-spun linen which was sewn in such a way that nothing might escape. This packet was closed by a cord whose ends were furnished with the seal of the examining magistrate of the court of Chateau-Thierry. To this packet was attached a tag on which were found the words: *clothing found on constable Hochet*.

The integrity of the official seals having been confirmed, the packet was opened and the clothing extracted. The packet contained: 1) a waistcoat of goathair in stripes and small colored points; this waistcoat which at first had a *water-green* color, had become *yellow* through use. This garment was saturated in blood in almost all its parts, particularly on the back, the neck and toward the pockets.

In one of the pockets of this waistcoat was a knife whose blade was stained by some white matter. Examination of the white matter staining this blade identified it as coming from crumbs of soft bread; indeed, a part of this white matter, separated from the blade burned with the smell of *roasted bread* when placed on live coals. Its volume increased on

^{*}Translation of: "Taches de Sang. Rapport Médico-légal".

in Annales d'Hygiène Publique et de Médecine Légale 14: 349-370 (1835).

contact with water. Finally, it took on a violet color on treatment with tincture of iodine.

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On some portions of the waistcoat a flaky white matter was noted. By the manner in which it behaved on being placed on live coals, this matter is comparable to the residue of potato pulp from which the starch was extracted.

2) Grey pants, where three openings, apparently made by a very sharp, cutting instrument, were noted on the waistline on the back to the right of the seam. These pants were saturated with blood on the waistline and near the openings and the surrounding parts. Selvaged suspenders also saturated with blood were attached to the pants.

The same substance, analogous to extracted potato pulp, was noted on the pants by the experts. The presence of this matter has to be explained. It appears that this white matter existed in the place where constable Hochet succumbed or that it can be found in the region where he was carried after his death.

3) A shirt of thick cloth soiled by a large amount of discharged blood; this blood was most appreciable on the back.

On the back part of the shirt, toward the area corresponding to the right kidney, four openings were noted, which were made with a sharp, cutting instrument. The place occupied by these openings is a certain indication they were produced by the same instrument which had pierced the waistline of /354 the trousers. The dimensions of the gashes demonstrates that

the instrument was a thin blade. 4) A pocket handkerchief in one of the corners in which is a knot containing two coins; one of silver, a franc, the other of alloy, a sou with the portrait of Louis XVI.

5) A constable's badge bearing the words: The law, department of Aisne, Pierre Hochet, rural constable of Lacroix, 1833. This badge was attached by an armband of linen.

6) An old cap of blue cloth with a copper visor. The copper is green beneath.

7) Selvaged suspenders, the half of which are stained with blood. The same flaky white matter previously mentioned is noted on the suspenders.

The presence of blood on the clothing of constable Hochet was sufficiently evident to us. Nevertheless, we considered it necessary to test a portion of material removed from the shirt. This material removed, it was separated into small fragments which were then reunited with pins, then placed in distilled water; after a few moments numerous striations were visible. The water colored in its lower part and gave rise to a brownish-red liquid, comparable in color to old, liquid blood.

After a suitably prolonged maceration, the liquid was separated from the fragments of material and divided into two parts, one of which was again divided and submitted to the following experiments:

/355 1) A quantity of this liquid was introduced into a glass tube, closed at one of its ends, and subjected to heating. The liquid soon became cloudy and presented a coagulum of

greenish grey. Treatment with potassium dissolved the coag ulum and the liquid resulting from the dissolution wa greenish-brown as seen by reflection and brownish-red b refraction, particular characteristics indicating that the solution, treated with heat, contained blood.

2) Another portion of this liquid treated with gall nu gave a coagulum of reddish-grey.

3) Another portion of the bloodied water treated with chlorine took on a green color, which disappeared with a excess of chlorine.

4) A portion of the liquid treated with a large excess o alcohol promptly deposited a lumpy precipitate of a splendie roseate-red color. These experiments demonstrated in the most evident way that it was actually dried blood staining this shirt.

A large quantity of this bloodied water, loaded with blood principles, was treated with pure sulfuric acid (of 66°), the stirred with a glass rod. The mixture was hardly complete when a strong odor of human sweat was emitted, an odo difficult to confuse with others.

Examination of the material contained in a sandston pitcher. The sandstone pitcher, about a pint and a half in volume, was removed from the case containing it. It wa closed with a paper acting as a label on which were th words: sandstone pot containing bloodied earth, moss leaves and pebbles found in the woods of Mesnil.

The pot opened, its contents were identified:

1) moss, a small amount of which was stained by a dry brownish-red substance, which appeared like dried blood

2) Earth, of which a few small portions were colored b a blackish brown matter, a color attributed to dried blood.

3) Pebbles, two in number, evidently bearing traces o dried blood.

4) Leaves of trees of which a few were soiled by a brown red substance, comparable to dried blood.

All the substances contained in the pitcher had an ex tremely strong *musty* odor, or better, of rotten wood, an odo due to the fact that all the substances were moist whe placed in the pitcher.

The portions of the different substances, moss, earth leaves, pebbles, which were stained and had acquired brown color, were separated from those which had not, and were put aside to undergo the following operations:

The moss was converted into a small bundle and placed i distilled water and left to macerate for a suitable length c time. The water situated in the lower part of the experi mental glass acquired a reddish tint. When the maceratio was sufficiently prolonged, the moss was removed and th red solution was divided into two parts. One was introduce into a glass tube closed at one of its ends. This solutior which had the same musty odor as the moss, and just a intense, presented the following phenomena when subjecte to heating. The liquid changed its color, became cloudy an gave a rather considerable coagulum of a rose-grey color Treatment of this coagulum with potassium dissolved it giving a greenish-brown color to the solution seen b

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reflection and a red-brown color by refraction. All these characteristics demonstrated that the examined liquid contained blood.

The other portion of the reddish liquid was treated with pure sulfuric acid (of 66°). A peculiar odor developed, but this odor was masked by the odor of rotten wood, which prevented the experts from recognizing the primary odor.

The leaves were then treated with distilled water to which they imparted a red color. The solution resulting from this maceration was examined; it was determined as containing blood. But the small quantity of this liquid, and the odor of rotten wood exhumed by the leaves, as well as the odor particular to the leaves, prevented the experts from experimenting further with the purpose of identifying the odor released by the reaction of sulfuric acid on this solution.

The pebbles were then washed with distilled water which received the coloring substance soiling them. The experiments performed on the colored water with heat demonstrated that this water prevented the experts from treating it with sulfuric acid for the purpose of developing the volatile principle of blood.

Finally, the portions of colored earth were treated with distilled water which colored it red. After division of the solution into two parts, one was suitably treated with heat. It presented all the characteristics indicating the presence of blood, i.e., there was coagulation, formation of a coagulum which redissolved with potassium, giving place to a greenish-brown liquid seen by reflection and reddish-green by refraction.

The other part was treated with sulfuric acid; but it presented a volatile matter having the musty smell or odor of moss.

Examination of a piece of blue cloth. This piece of cloth bore a tag on which could be read: piece of cloth serving as material evidence in the Hochet affair. Examination of this material demonstrated it was about six feet in length. It appeared to come from an old slip, so worn out that at first glance it appeared covered in flour, which it wasn't, as our experiments demonstrated.

The remains of this garment were patched up several times with patches of different qualities and colors.

Thirty stains, apparently due to blood, were noted on this piece of cloth which presented these spots strewn on a blue background. A few of these stains were very large, about two thumbs square. Various remains of dry vegetable matter were attached to this piece of cloth, which were identified as strands of straw, hay and stems of mustard-seed.

The greater part of these stains, found on the remains of this garment, were removed and placed in distilled water which colored in red-brown. A part of the bloodied water thus obtained was tested with heat and various reagents and presented all the characteristics of liquid blood.

Another part of this water, treated with sulfuric acid, developed a volatile principle which was identified by one of the experts as having the odor of woman's menstrual discharge, and by the others as having the odor of human

excrement.

Examination of the clogs of Jean-Baptiste Boileau. Exam- 360/ ination of the clogs demonstrates they were worn for quite a while. Their interior was filled with earth which had accumulated under the form of mammilate plaques. This earth presented no coloration attributable to blood.

Examination of the exterior identified a stain of blackish color on the inside angle of the heel of the right foot. This stain, which had the form of a diamond, was about one thumb square. Beside this stain were different materials attached to the clog, among which were distinguished straw, earth, sand, etc. On the clog of the left foot, various points colored in violet-red were distinguished on the outside in front. Finally, stains made from grass were visible on the bottom of the clogs but none of these stains could be attributed to blood.

The black stain in the form of a diamond found on the heel of the clog of the right foot was removed by scraping with a penknife. The parts scraped off were placed in distilled water; after a rather prolonged stay, the water, which had dissolved the soluble elements, was examined. As a result of this examination it was determined that the stain on the clog was not due to blood. Indeed, this stain, of a yellowish color, emitted a foul odor of excremental matter. Exposed to heat, 361/ it didn't cloud nor furnish a coagulum. The odor emanated indicated that this stain was actually due to fecal matter.

The other material, straw, earth and sand, which had been found near the stain, and which formed a slight elevation, were removed and examined. By similar means, it was found that, as in the case of the previously examined stain, these materials had been fixed to the clog by the fecal matter.

The stains of a violet red, found on the front part of the clog of the left foot, were removed along with a part of the wood of the clog and placed in distilled water for maceration and left in this liquid.

The resulting solution did not acquire a reddish color. Tested by heat, it furnished no characteristics indicating presence of blood.

Examination of the trousers of Jean-Baptiste Boileau. Scrupulous examination of all parts of this garment revealed to the experts:

1) In the pocket on the right side of the waistline a stain apparently due to blood and whose position indicated it as having been made from the back of a hand.

2) On the lower part of the right leg, in front, three stains apparently made by droplets of blood projected onto the pants. These droplets did not pass through the material.

3) On the same lower front part of the right leg, three other stains, apparently due to blood. These stains had a tint different from the first and this is an almost certain indication that they are of an earlier origin than those previously described.

4) A bit below the knee of the left leg a stain, apparently due to dried blood. This stain had all the characteristics of a stain much older than some of those of the right leg.

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5) On the lining on the right inside a blood stain could be noted. This stain, like that observed on the pocket, seems to indicate that Jean-Baptiste Boileau was wounded on the back of the hand and that he carried the wounded hand in his pants and in his pocket.

6) On the back of the pants, different stains, but which were not due to blood.

About half of the fabric bearing the stain found in the pocket was removed and placed in distilled water. After remaining in this liquid for a certain amount of time, it imparted a roseate tint to it, as a small amount of blood would do. The water, impregnated with soluble elements of the stain, changed color when heated in a glass tube, became cloudy and presented a coagulum which redissolved with potassium with the characteristic phenomena indicating the /363 presence of blood.

One of the three stains, which appeared less old than the others, was also removed and treated in the same manner. It presented a roseate liquid which gave the characteristics indicating the stains were due to blood.

Examination of the smock of Jean-Baptiste Boileau. This smock, of thick blue cloth, was designated under the name of a smock with two sides. However, only the side which could be considered as being the front could be distinguished on this garment.

Examination of this smock revealed to the experts:

1) On one of the sides, designated by an "A" traced on the smock, thirty stains, presenting characteristics of decay. Physical examination of these stains did not permit us to estimate the nature of the substance which had produced them.

2) On the sleeve of the right side when facing the side "A", twenty stains apparently from the same moment as the preceding. No stains were noted on the left sleeve.

3) On the other side of the smock, three stains on the left /364 sleeve and four on the right sleeve. All these stains seemed to have the same origin.

A rather large number of stains were removed from the smock, along with the fabric, and placed in distilled water for more than four hours. The water had not acquired a reddish color at the end of this time, but a yellow color. Heated, this water didn't cloud or present a coagulum, which indicates these stains were not made from blood.

The portions of fabric bearing these stains were removed from the water and left to dry. After drying they were treated with boiling, 40° alcohol. This alcohol colored in green and the filtered alcohol solution became extremely cloudy with water.

After evaporation to the point of dryness of a portion of the alcohol solution by low heat, a material of resinous nature very comparable to glue remained. This matter, which formed the stains noted on the smock of Jean-Baptiste Boileau was green, adhered to fingers, causing them to stick together, had a bitter odor, and emitted aromatic smoke of a peculiar odor on burning. The experts believe these stains to have been produced by glue.

Examination of the (old) smock of Victor Darez. Examination of this smock revealed that it contained stains or various parts which we could not attribute to blood by their color. To be assured of their nature, however, they were removed from the smock and placed in distilled water. These stains did not color the liquid in red, but communicated a yellowish color to it. Submitted to heat, the water did no cloud and furnished no coagulum. It presented none of the characteristics belonging to water impregnated with blood

After drying the material bearing these stains, and treat ing it with boiling, 40° alcohol, it gave an alcoholic solutior which left a certain amount of fatty matter when evaporated to dryness. It was this fatty matter which formed the stain: noted on the smock by attracting dust.

Examination of the new smock of Victor Darez. Examination of this garment revealed it was two-sided. On one of them, marked "A" by the experts, a large number of stains were noted which, with the exception of one, which we surrounded with a square traced in ink, were not made by blood but by fatty matter. As for the stain enclosed in the square it appeared to the experts to be due to blood and was preserved, its presence being the only fact which could later prove that this smock was stained by blood.

On the other side of this smock were noted:

1) A large number of stains in various places which were produced by fat.

2) On the right arm, about forty stains which the experts believed due to blood. These stains appeared to have beer made by spurting blood; at least, their disposition seemed to imply this. They didn't appear to be very old, to judge by their color, and the shiny appearance which they had preserved.

A portion of the stains we suspected of being made from fat was removed with the fabric and placed in distilled water But this liquid did not color in red, and the water didn' change color nor become cloudy after being subjected to the action of heat.

Dried and treated with boiling, 40° alcohol, the fabric bearing the stains presented a fatty matter which had caused the stains.

A portion of the stains on the sleeve of the new blouse of Victor Darez, stains which the experts considered as being made by blood, was removed along with the fabric and placed in distilled water, which colored in rose. The wate: thus colored became cloudy when subjected to heat in a glass tube closed at one of its ends. It then gave a coagulum which redissolved with potassium. At the same time, the solution presented a greenish color in reflection and a reddish color ii refraction. These characteristics indicate that the wate: tested contained dissolved blood coming from the stains.

Examination of the shirt of Victor Darez. The exam ination of this shirt revealed:

1) On the bottom of the lower part of the front, small blood stains.

2) On the upper, internal part of the right sleeve, toward the middle of the sleeve, four stains, of which two appear to

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be of blood.

3) On the back part of the left sleeve, near the wrist and a bit above, five stains apparently due to blood.

Several blood stains were also noted in the inside of both tails of the shirt: 1) on the lower part of the back tail; 2) on the front tail corresponding to the pubis. The form and disposition of these stains seem to indicate that the wearer of the shirt had pursued the act of coitus with a woman during her menses. Tests performed on part of the stains found on the sleeves of the shirt by distilled water and heat demonstrated they were due to blood.

The stains found on the shirttail furnished, with distilled water and heat, results indicating that these stains were also due to blood. The experts did not make any attempts to determine the aroma of the blood for it was found in very small amount on the shirt and, besides, they emitted a very strong odor which would undoubtedly have masked that specific to the blood.

These procedures finished, the experts found it possible to reply to the questions posed only in the following manner: For the first question:

It was impossible to determine if the blood mixed with earth taken from the woods of Mesnil is blood of man or from the same man as that found on the clothing of Hochet, on the clothing of Jean-Baptiste Boileau and on those of Victor Darez for the reasons: 1) the blood was in too little quantity; 2) the blood found on earth and moss taken from the woods of Mesnil had contracted a strong odor of decaying wood which had abolished the odor peculiar to blood no matter from what source it came.

For the second question:

It was possible to identify the blood stains on the trousers of Jean-Baptiste Boileau, but it is impossible to determine if this blood is of man or of hare or if it is from the same man as that found on the clothing of Hochet or that found on the earth taken from the woods of Mesnil. This impossibility is explained by the very small amount of blood found on the trousers of Jean-Baptiste Boileau. As for the question of determining if the blood found on the trousers was there for three weeks or four months or more, the experts claim that the blood stains observed on these trousers were produced at two times, evidently different from each other.

For the third question:

It was possible for the experts to determine the presence of stains made by blood on the new smock of Victor Darez and on his shirt. But it was impossible for them, because of the small amount of blood, to say if this blood is of man or of sheep, or if this blood is the same as that found on the clothing of Hochet and on the earth from the woods of Mesnil.

As for the question of determining if this blood existed for the past three or five weeks on the clothing of Victor Darez:

It is impossible for the experts to say if the blood stains observed on the clothing of Victor Darez had an existence of three or five weeks. They are convinced that those seen on the upper part of the right sleeve, on the side of the smock marked "B," are of the same date as those observed near the seam attaching the sleeve to the smock. Although the former are less visible, they attribute the difference between them to friction undergone by the former and from which the latter were protected by the stitching of the seam.

For the fourth question:

It was possible for the experts to determine whether the stains found on a piece of blue cloth found near the place where the earth was taken from the woods of Mesnil are due to blood. But the experiments done with sulfuric acid, with the purpose of comparing the volatile principle released from the water which had dissolved the blood found on the clothing of constable Hochet, lead them to believe that the blood which had stained the piece of blue cloth is not the same as that staining the clothing of the constable. Indeed, the volatile principle released from the water impregnated 370 with the blood of Hochet did not resemble that released from the water in which the piece of blue cloth was immersed and was not the same.

Memoir on a New Method for Recognizing Blood Stains*

M. J. B. Orfila

About six years ago, M. Persoz, professor of physics at the science faculty of Strasbourg, informed me that, in 1836, he resorted to hypochlorous acid for the recognition of blood stains on a smock where urine stains were also found. "This acid," he said, "destroys immediately every stain except those formed by rust or blood: the latter turn a blackish brown on contact with the acids. It is that much more important to make use of hypochlorous acid since it often happens that blood stains found on these fabrics lose the property of dissolving in water and consequently cannot be determined by this method."

A little while after this communication, I applied jointly with M. Cottereau, the method indicated by M. Persoz, in a medico-legal analysis, on the occasion of a confinement pending trial for murder. It was a matter of determining whether the shirt worn by the murder victim, and a smock and a scythe seized at the home of the accused, were stained by blood or not. After having treated these stained objects with distilled water, and having exhausted the series of char-

/113 acteristics I had indicated in 1826 in my memoir on blood stains, we resorted to hypochlorous acid and we determined: 1) that this acid applied on a region of the blue smock where there was no type of stain discolored and whitened this region in an instant; 2) that the dotted stains of brown red, which existed on the smock, resisted the action of the acid and acquired a darker color; 3) that the stains of the scythe blade furnished nothing with distilled water, that they completely dissolved in hydrochloric acid, and were in no way modified by hypochlorous acid.

Prior to this assessment, I was consulted by MM. Magonty and Loust, pharmacists of Bordeaux, who were charged by the Public Prosecutor with determining the nature of certain stains found on the lining of a coat pocket. These gentlemen, having met with a few difficulties in resolving the problem by means known up to the present, asked me to indicate a procedure specific for ridding them of these obstacles. I immediately replied to them that they could use hypochlorous acid, and furnished them with all the information which could guide them in their research. One will see by the letter which I transcribe with what shrewdness these experts operated in this circumstance.

Letter of MM. Magonty and Loust

To Monsieur Orfila, Dean of the Faculty of Medicine of

Monsieur le doyen,

We would have already thanked you a long time ago for your kindness in replying to us, in showing us a method entirely new to us, a priceless method for determining the presence of blood on fabric, if we had not had to repeat this experiment and commit ourselves to rather meticulous research which was the consequence. Today, now that our work is finished, we are eager to inform you of the cause of our long silence; and in asking you to excuse it, we must express our warm gratitude to you and confide to you the results which we have obtained.

To confirm the facts which you made known to us, not that we doubted their verity, but as an indispensible exercise, we did many practice procedures, operating on fabric stained by us. We assured ourselves that the hypochlorous acid, prepared with chlorine which one hadn't taken the precaution of washing beforehand, gave uncertain results. The stains, in effect, after a half hour of maceration, became very pale without disappearing entirely, however. But the same reagent, washed of hydrochloric acid, behaved as you told us; only, after a prolonged maceration of a few hours, the stain, which at first darkened and browned, became a bit more pale, but did not disappear.

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We considered ourselves sufficiently informed, and we undertook to do the legal research which the examining magistrate demanded of us. We were not a little surprised to see the stain disappear in great part; however we noticed *brownish lines* which persisted in the manner of blood stains.

We ask you to recall the nature of the report which we had the honor to submit to you; we had to report on the nature of stains found mainly on the lining of a coat pocket; and we say that the physical characteristics of these stains caused us to believe *that they did not come* from a spurt, but from contact with a stained object.

We had to investigate if there were not a difference between *direct* stains, i.e. those coming from spurt of blood and the immersion of fabric in this fluid, and stains we will call *secondary*, i.e., those produced by contact with a stained body.

There should be, in effect, a chemical difference between direct stains receiving all the elements of blood, and secondary stains produced by them where the beginning of coagulation must have fixed the elements retained by the *clot* on the first fabrics. To assure our-

^{*} Translation of: "Mémoire sur un nouveau Moyen de Reconnaitre les Taches de Sang."

in Annales d'Hygiène Publique et de Médecine Légale 34: 112-129 (1845).

selves of the degree of credibility we should give to this idea, we wanted to perform comparative experiments with direct stains, secondary stains, and those acting as subject for our research. We consequently stained white linen with blood coming from a patient's vein, and a few seconds later we pressed the first linen on a portion of red fabric cut from another pocket of the accused's coat, fabric similar in every point with that of the incriminated pocket. We designated these linens by numbers 1, 2 & 3. We placed three approximately equal pieces of the linen in three glasses containing hypochlorous acid and we were able to determine: 1) that the direct stain (no. 1) behaved as you had informed us; 2) that the secondary stain and that of the pocket (nos. 2 and 3) partially disappeared, both in the same manner, i.e. the threads of the woof, more pronounced than those of the warp, and which must have absorbed more of the liquid on contact with the stained object, conserved a brownish imprint, whereas the threads of the warp were discolored.

We wanted to investigate further this resemblance of numbers 2 and 3 provided by hypochlorous acid in the first method we had used. We then started the whole operation over in comparing secondary stains with the pocket stains; we constantly obtained the same results . . . and from this moment our doubts ceased.

Thus, sir, thanks to your kindness we have been able to use a reagent new to us, of which we have been able to confirm the consistency, and which should henceforth render great service.

It was also demonstrated that there exists a notable difference between direct stains of blood and secondary stains, a difference of which we feel experts in legal medicine should always be aware.

We will terminate this letter as we began it, in asking you, sir, to receive expression of our gratitude and to consider us your very respectful students.

> M. Magonty and Loust. Bordeaux, January 18, 1842.

I thought it necessary to examine the question carefully, to find out exactly if hypochlorous acid offered the advantage associated with it by M. Persoz; for this I attempted a great number of experiments.

Action of liquid hypochlorous acid on blood stains¹

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First Experiment. A white cloth stained by blood flowing from a vein is immersed in liquid hypochlorous acid; at the end of *thirty seconds* the cloth is retrieved and it is seen that the stain is browner than before immersion; *seventeen hours* after being exposed to air it retains the same color.

Second Experiment. A white cloth stained with blood by applying it to a thick stain from a *spurt* is left in liquid hypochlorous acid during thirty seconds. On being removed from the liquid, the stain is a light brownish toward the middle, and almost uncolored at its circumference: exposed to air, this part continues to discolor; however, the points on this circumference where the stain was a bit thicker were light brown. At the end of *seventeen hours* the cloth is dry and presents a *greyish* tint there where the blood stain was first found.

Third Experiment. A white cloth stained like the preceding is immersed in liquid hypochlorous acid; at the end of *three minutes*, the points weakly colored by blood are discolored; after *ten minutes* of contact there remains on the cloth only three small greyish plaques. *Ten minutes* afterwards, two of these plaques are completely discolored; *forty minutes* afterwards the last of these plaques has disappeared.

Fourth Experiment. Linen is stained with poppy oil, then a *thin blood stain* is made on this linen. After *thirty seconds* of immersion of this linen in liquid hypochlorous acid, the stain is red brown, and shows no change on prolonged exposition to air. But if the linen remains immersed in the acid for an hour, the stain disappears entirely.

Fifth Experiment. White linen with blue stripes stained *two years* ago by a small amount of human blood is immersed in liquid hypochlorous acid. Removed at the end of *thirty seconds*, it is almost entirely discolored; whereas the stain still presents a hint of clear red, although it obviously 118 tends to disappear. After *twenty four hours* of exposition to air, all remains in the same state. The same results are obtained with white linen with blue stripes stained *six years* ago with a rather large amount of blood.

White linen covered *two years* ago by a *thin* blood stain is immersed in hypochlorous acid for *thirty seconds*; the stain blanches; after another immersion of a quarter of an hour, only a dirty grey tint is seen on the stained part.

A piece of black sheet presenting a rather thick stain of human blood, *two years* old, is in part discolored after *thirty* seconds of immersion in hypochlorous acid, whereas the blood stain is darker and almost black. After a *twenty-four* hour exposition to air, the sheet is less colored, and the stain conserves its black color.

Sixth Experiment. Pieces of black sheet, grey duck, red calico and white cloth, stained *two years* ago by human blood are left for *two minutes* in liquid hypochlorous acid; with the exception of the red calico, which presents only a thin blood stain, the other fabrics are stained by a rather thick layer of blood. On removal, all the fabrics are discolored, while the blood stains have become a blackish brown. After exposition to air for *twenty-four hours*, all remains the same.

Seventh Experiment. Some coagulated pigeon blood is diluted in a little water and part of the liquid is applied on white linen striped with blue. After a *few seconds* of immersion of the linen in liquid hypochlorous acid, the linen is discolored and the blood stain, red beforehand, has already

¹ Hypochlorous acid was prepared by the method of M. Ballard, in shaking mercury dioxide diluted in water into *perfectly washed* gaseous chlorine; the liquid was filtered at the end of the reaction, and used in this state, as did M. Persoz.

/119 acquired a fallow tint. Six hours after retrieving the cloth from the liquid, the stain was of a hint of fallow, so clear that it was almost entirely discolored.

No change had appeared the following day.

Eighth Experiment. A blood stain, recently made by letting venous blood flow on white linen, was immersed in liquid hypochlorous acid for six hours. At the end of six hours the stain was still a brownish black. After seventeen hours of immersion, the linen reduced to a pulp when pressed by the fingers and only a clear gray plaque was perceptible in the stained area.

Ninth Experiment. White linen, stained two years ago by human blood, is left for six hours in liquid hypochlorous acid. The stain passes to a deep fallow, then clear fallow, and this nuance is such that it perfectly resembles stains made by alkanet and a fatty matter after six hours of contact with hypochlorous acid. All were in the same state the following day.

A piece of black sheet, also stained two years ago by human blood, after having remained in the same acid for six hours, was retrieved completely discolored; the blood stain had also lost its color, however its discoloration had happened more slowly than that of the sheet.

Tenth Experiment. Pieces of black sheet, of gray duck, of red calico and of white cloth, stained by blood two years ago, are immersed in hypochlorous acid. At the end of two hours, these materials are completely discolored; but the blood stains are blackish and do not look as if they are disappearing. After sixteen hours of immersion, the red calico presents a very clear cafe au lait tint in the area of the stain; the duck is a brownish green with stained points, where whitish debris are seen, probably coming from the action of

/120 hypochlorous acid on the blood; the white cloth preserves only two bulging black points of the stain, which are the size of the eye of a needle; finally, the sheet, now a greyish brown. covered in whitish debris, presents three rather thick stains, black in the center, whitish yellow at the circumference.

Eleventh Experiment. A blue-and-white striped material, stained six years ago by a rather large amount of human blood, left for six hours in liquid hypochlorous acid, is discolored on being removed, and the blood stain is of an excessively clear fallow color.

Twelfth Experiment. An iron blade presenting a thin blood stain, recently made, is immersed in hypochlorous acid. At the end of thirty seconds, there remains only a brown red tint at the stained spot; during the action of the acid, a rather large quantity of gaseous chlorine is released, and red iron oxide is formed. After an hour of immersion in the same acid, the blade is covered with a rather thick layer of iron sesquioxide and, if this is removed by a thin trickle of water, the brown-red tint of which I have spoken is perceived at the area formerly stained by blood. The blade is immersed in another portion of hypochlorous acid. After five hours of immersion, the iron is once again covered by a thick layer of oxide, but if this is detached by a thin trickle of water, or by lightly rubbing with wet tweezers, small stains of red brown

are once again noted here and there: one would say that iron oxide and the portion of blood remaining on these points forms a mixture producing these stains.

Thirteenth Experiment. After six hours of contact with hypochlorous acid, an excessively thin blood stain, recently made on an iron blade, has completely disappeared, and the metal is thoroughly scoured.

Fourteenth Experiment. An iron blade presenting two thick recently made blood stains is left for thirty seconds in hypochlorous acid; chlorine is given off and iron oxide produced; the stains are a reddish brown. At the end of one hour they conserve the same color, but detach in parts at a few points and the iron presents its normal sheen. The blade is then immersed in a new bath of hypochlorous acid. After six hours of contact, the stains are still brown in the center; their circumference, a dirty red, shows a kind of rim formed by iron sesquioxide. After fourteen hours of immersion, one of the stains is a greyish white and encrusted with iron oxide; the other is reddish brown, detaching in plaques; in leaving the blade in hypochlorous acid for thirty-eight hours, it is largely covered in iron sesquioxide and the liquid contains a large amount of iron sesquichloride; when this oxide is removed by a thin trickle of water, the stain which had remained is reddish-brown color, still presents the same tint, but is held to the blade only by a few points at its center.

Action of hypochlorous acid on stains produced by various colored materials

Fifteenth Experiment. Material stained in black by fat and coal is left in hypochlorous acid for twenty-four hours; the stain undergoes no alteration.

Sixteenth Experiment. Blue material recently stained by alkanet and fat is promptly discolored, while the stain remains. After six hours of contact, the stain, which had been a dark red, acquired a fallow tint, similar to that taken by the blood stain considered in the ninth experiment, p. 119.

Seventeenth Experiment. Material covered for a month by a large and thick stain of alkanet and fat is immersed in hypochlorous acid. The material promptly discolors, but the stain, at first a blackish red, has acquired at the end of two hours the color of rust. This color has lost none of its intensity after sixteen hours of immersion.

Eighteenth Experiment. A portion of the same material presenting a large, thin stain, of the same nature as the preceding, discolors almost instantaneously; at the end of two hours the stain is a fallow yellow. This tint has lost none of its intensity after sixteen hours of immersion.

Nineteenth Experiment. Blue material on which is found a thick stain of alkanet and fat is immersed for a few seconds in hypochlorous acid and discolors, while the stain remains red.

Twentieth Experiment. Blue material stained a month ago with a mixture of fat and alkanet is left in the same liquid acid for thirty seconds. The material is completely discolored, and the stain, which was thick and red brown, shows

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a rusty color toward its circumference and blackish at its center. This remains about the same after *sixteen hours* of exposure to air.

Twenty-first Experiment. This experiment is repeated with a large, *thin* stain made a month ago with alkanet and fat; the stain, reddish before immersion, is a fallow color on removal from the liquid. The stain retains a very clear, reddish yellow color after *sixteen hours* exposure to air.

Twenty-second Experiment. White material stained in red by a mixture of madder-wort and poppy oil is left in hypochlorous acid for a *few seconds*; the stain persists.

Twenty-third Experiment. A part of this same material is immersed in hypochlorous acid for *five hours* and the stain covering it just about conserves its color; the following day a portion of the stain is completely discolored; the day after there no longer remain traces of the color.

Twenty-fourth Experiment. After thirty seconds of immersion in hypochlorous acid of white material stained by celandine, the thin stains are yellowish and the thick brownish at their center. After *five minutes* of immersion, all the thin stains have disappeared, and rust-colored circular lines, which end up yellow, replace the thick stains.

Twenty-fifth Experiment. White linen colored like the dregs of clear wine by *campanula pyramidalis* is immersed in hypochlorous acid; at the end of *thirty seconds*, the stain disappeared.

Twenty-sixth Experiment. White material colored here and there by clear bister and brown in a few places with *taraxacum dens leonis* is left in hypochlorous acid for *thirty seconds*; the rather thin stains of a dark-brown color are entirely destroyed; the others, four in number and thick, are almost completely discolored; at the end of *thirty minutes*, there remains no trace.

Twenty-seventh Experiment. Stains made on white material with *cichorium intybus* are of a *very clear* bistre; those which are thin entirely disappear after *thirty seconds* of immersion in the acid; the thickest are discolored at the end of *ten to twelve minutes*.

Twenty-eighth Experiment. White material stained a reddish brown by *lactuca virosa* is immersed in hypochlorous acid; at the end of *thirty seconds* the thin stains have disappeared; the thicker ones show a yellow color; the brownest of 4 the thick stains is rust colored. The material is exposed to air for a quarter of an hour, then again immersed in acid; at the end of ten minutes the stains changed to yellow are discolored; ten minutes later, the rusty stain is a lightly yellowish white.

Twenty-ninth Experiment. White material stained a very clear reddish brown by *euphorbia lathyris* is discolored in a few minutes by hypochlorous acid.

The various stains employed in experiments 24, 25, 26, 27, 28 and 29, were done August 21, 1842.

Thirtieth Experiment. White material is stained red with a mixture of fat and *colcothar* (anhydrous iron sesquioxide): the stain undergoes no alteration, even after several days of contact with liquid hypochlorous acid.

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Thirty-first Experiment. White material stained like the preceding and not discolored by hypochlorous acid, is put in contact with a mixture of tin protochloride and hydrochloric acid, as recommended by M. Persoz. The stain blanched after a quarter of an hour; *seventeen hours* later, it had almost entirely disappeared for there remained only a few excessively clear red points bordering on yellow.

A stain of *blood* as thick as the preceding had undergone no change, even after several days, with tin protochloride and hydrochloric acid.

Thirty-second Experiment. White material is stained by a mixture of fat and *colcothar*; the stain is then covered with oil. The material is immersed in a solution of *tin proto-chloride slightly acidified by hydrochloric acid*. After three days of contact, the solution is excessively cloudy, and *the stain persists with no change*.

Thirty-third Experiment. Rusted iron is not discolored in liquid hypochlorous acid even after six hours of contact.

Thirty-fourth Experiment. Iron, stained by a mixture of fat and madder-wort, is not discolored after *six hours* of contact with liquid hypochlorous acid; but it is the following day.

Thirty-fifth Experiment. Iron, largely stained with a mixture of *colcothar* and fat and not discolored by hypochlorous acid, is put in contact with a mixture of tin protochloride and hydrochloric acid. At the end of twenty-four hours the stain disappears and the iron blade regained its sheen.

Action of water on blood stains

Thirty-sixth Experiment. A piece of black sheet is stained with a mixture of poppy oil and human *blood*. The following day the sheet is put in water; at the end of a few minutes the liquid is rose-colored and it can be confirmed that it has the characteristics of the coloring matter of blood. Stained material likewise provided a rose liquid after *ten minutes* immersion in water, giving an appreciable quantity of coloring matter of blood.

Thirty-seventh Experiment. Thin and thick stains of blood were made on material and on sheets covered with fat beforehand. Other pieces of the same material and same sheet were first stained with blood then covered with a light layer of fat. The following day the various fabrics were put in water, and at the end of a few minutes, it can be shown that they give off an amount of coloring matter to the water, such that this substance can be easily identified by the action of *heat*, chlorine and the other agents which I recommended use in my public memoir of $1826.^2$

Thirty-eighth Experiment. A small piece of white material, stained six years ago by a rather large quantity of

² This procedure has already survived the test of time; during 20 years there has not been an assessment of blood stains that has not made use of it; all those who have recently written on legal medicine adopted it without modification, to begin with by M. Devergie who presented it verbatim in his work, without indicating the source from which he had borrowed it and without even mentioning my name.

human blood, is put in contact with a gram of water. At the end of a *quarter of an hour* the liquid yellows and tends to acquire a rose tint similar to that which water gives to a very small amount of blood; the heated liquid foams and coagulates; chlorine and the other agents behave with it as with a solution diluted with coloring matter. If another portion of this material is left in distilled water for *twenty-four hours*. the liquid acquires a color a bit more intense, evidently bordering on rose, and it undergoes the same changes with heat as had been obtained after a quarter-hour contact. The material stays red.

Action of water on stains produced by various colored materials

Thirty-ninth Experiment. Material stained by chelidonium majus, campanula pyramidalis, taraxacum dens leonis, cichorium intybus, lactuca virosa, and euphorbia lathyris, was put in contact with distilled water, which colored pale yellow, brown, or blackish brown. These different liquids, heated to boiling, retained their colors and did not coagulate.

Conclusions

1) Of all the methods proposed up to the present for the recognition of blood stains, that consisting of treatment of the stain with water and then working with the solution, as I recommended in 1826, is undoubtedly the best. M. Persoz

/127 is evidently mistaken when he claims it often happens that blood stains found on fabrics lose their property to dissolve in water and cannot consequently be disclosed with the help of this liquid. The hundreds of assessments performed up to the present, and experiments 36, 37 and 38 reported in this memoir, establish, to the contrary, that in almost all cases, blood stains, even very old, made on clear material or coated with fatty bodies, or on iron, give off coloring matter to the water in quantity great enough for blood to be easily recognized. Moreover, numerous experiments, which I undertook in 1826 and results of which I related in this memoir (cf. exp. 39), demonstrate that all coloring substances, without exception, other than blood, applied on materials, produce stains behaving in water otherwise than do blood stains.

2) Hypochlorous acid is far from having the advantages indicated by M. Persoz; experiments 1-14 described in this memoir demonstrate that the greater part of blood stains, thin or thick, recent or old, on material or on iron, entirely, or almost entirely, disappear after a stay a bit prolonged in

hypochlorous acid; that if some of them don't completely disappear, far from being brown red, they leave only a greyish tint. To tell the truth, some of these stains, even though disappearing almost totally, conserve a brown red color in their center.

In accordance with what was said by Mr. Persoz, if the action of hypochlorous acid is not prolonged for more than a few seconds, one or two minutes, the blood stains persist and turn brown, even though dried-out and old; but, on the other hand, stains from a mixture of alkanet and fat or fat 1 and charcoal or madder-wort or poppy oil or with chelodinium majus, etc., behave a bit like blood stains in hypochlorous acid; it is, therefore, impossible to positively characterize the nature of a stain by the action of this acid only, even if the immersion of the stained parts is of short duration (cf. experiments 15 and 24).

3) However, if hypochlorous acid is insufficient for positively establishing that a stain is formed by blood, it can be used with some advantage as an accessory method, provided it remains in contact with stained material only for one or two minutes at most; if there exist some coloring substances other than blood, which behave somewhat like the latter with this acid, the stains produced by these matters, even though persisting, do not acquire precisely the same tints as blood; besides, the number of coloring substances which hypochlorous acid destroys in less than two minutes is great, while this time is insufficient for the acid to obliterate blood stains.

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4) Hypochlorous acid is completely useless for distinguishing *thick* blood stains on material or iron from rust stains or those produced from a mixture of colcothar and fat because these stains persist even after prolonged action of the acid. But if this is insufficient in this case to resolve the problem, one can successfully turn to the method proposed by M. Persoz, consisting of treatment of thick blood stains with a tin protochloride solution acidified by hydrochloric acid; the thick blood stain will resist, while rust stain and that produced by a mixture of colcothar and fat will disap-1 pear at the end of a few hours, provided that the latter is not covered by a layer of oil.

5) The action of hypochlorous acid on blood stains coming from a spurt of blood or by immersion of material in blood, visibly differs from that exerted on stains one might call secondary, i.e., those produced by contact with a body stained by a spurt; indeed, these latter resist much less than the others the discoloring action of this acid.

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Legal Medicine. Medico-legal Research on Blood*

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Chapter I. The Use of the Microscope in Medico-Legal Research

The first physician who tried applying the microscope in a /561 medico-legal assessment is, indisputably, M. Orfila. In 1827, this distinguished professor speaks of it in his work on blood (J. de Chimie Med., v. III; Paris, 1827, p. 413) and on semen (ibid, p. 473). One must regret that his research was not characterized by successful results. We will explain later (Chapt. II, § III) the circumstances which must have hindered M. Orfila from making use of his microscopical observations in recognizing different types of blood; it suffices to point out here that analogous circumstances were encountered in his research on semen. M. Orfila succeeded in identifying animalculi in dried semen on a slide 18 years old; but when he wanted to examine dried semen on linen by microscope, after having dipped the linen in water, he was led to the conclusion that the zoosperms were no longer perceptible.

M. Rattier (Journ. de Chimie Med., March 1837, p. 120) making some observations on linen stains, in a medico-legal context, saturated linen with water. The water washing the linen contained debris of spermatic animalculi and some whole animalculi. It appears, according to M. Rattier, that at the time of the Contrafato trial, Lebaillif was already using the microscope for identification of semen stains; but, for reasons we can hardly understand, his research was guarded with the greatest secrecy.

M. Ollivier (of Angers) was the first to apply the microscope practically in medico-legal expertise. In the month of June 1837, he was charged with determining if there did not exist hairs adhering to an axe seized at the home of an individual accused of homicide, and, if affirmative, to determine the color of the hair. M. Ollivier, with the help of a microscope, recognized that the filaments in question were fur, completely differing from hair, while perfectly resembling the fur of a horse, beef or cow, when comparatively examined; the judicial inquest fully confirmed the correctness of this observation (*Arch. génér de méd.*, Dec. 1838).

In the meeting of the 20 Nov., 1838, M. A. Devergie read

a note on the characteristics of hanging in a living man. He noted the presence of spermatozoa in the urethral canal. He claims, in addition, to have noted spermatozoa in seminal stains on linen ten months old. M. Devergie, however, pointed out that procedures specific for separating spermatozoa from the linen on which they were deposited very often altered them, in separating the tail, and of rendering microscopical examination not only difficult, but fruitless. Historical facts, which we have presented in chronological order, clearly demonstrate, it seems to us, that M. Devergie was going a bit far when he said of himself, "happy to be the first to introduce the use of the microscope in medico-legal research". (Ann. d'Hygiene Publique, Paris, Jan, 1839, p. 169). He appears to agree on this point himself later on (Ibid, April, 1839, p. 478). We do not feel it necessary to occupy ourselves here with the question of priority raised by M.M. Devergie and Bayard, since on the one hand, priority undisputably belongs to M. Rattier, and on the other, only M. Bayard is seriously concerned with microscopical examination of seminal stains, as we will later see. Let us add here that M. Donné, since 1837 (on spermatoza), noted the possibility of recognizing the presence of zoosperms after a more or less prolonged stay in urine. These findings were discovered in a physiological study, which does not hinder at all their application to legal medicine.

M. Gaultier de Claubry was charged, in June 1838, along with MM. Labarraque and Ollivier (of Angers), with a legal assessment, which had as its object the study of a large amount of denatured, adulterated opium; they proved by microscope not only the adulteration, but also discovered, by this means, the different methods of extraction of opium from Smyrna and from Egypt (announced in the previously cited work of M. Ollivier (of Angers), Arch. gén. de méd., 1838 and published in Ann. d'Hygiène, Oct. 1839, p. 374).

M. Bayard (Ann. d' Hygiène, July, 1839) did some further research concerning the microscopical examination of dried semen on linen or material of varied color and nature which were undertaken during the month of November, 1838. For the recognition of dried seminal stains on linen, and making use of microscopical observations, M. Bayard pointed out it was necessary to take care not to crumple or to separate the strips being macerated. Filtration of the liquid of maceration and examination of the deposits remaining on the filter reveal the presence of spermatozoa, isolated from mucus,

^{*} Translation of: "Médecine Légale. Recherches Médico-légales sur le Sang".

in Gazette Medicale de Paris 10(37): 561-567 (September 3, 1842).

complete, and without tearing of the tails. He could thus recognize semen dried out two months, two years and nearly three years before. The nature and color of fabric stained by semen has no bearing on the microscopical analysis and the recognition of spermatozoa; one finds them as easily in fabric of thread or of cotton as in that of wool or of silk. One can easily confirm the presence of spermatozoa in vaginal mucus taken after coitus.

Let us now permit ourselves a few reflections on the historical account just presented. It is evident that legal medicine could already have drawn much use from the microscope in many questions. Thus, certainly every time it is a question of determining the presence of sperm either on linen, in the vagina, or in urine, etc., one's recourse is necessarily the microscope as the sole specific means of resolving the question. We would be most satisfied to see the use of the microscope sanctioned in medico-legal research, not only in theory, but also in practice, by the approbation of forensic physicians who have not made a special study of the microscope, and who have consequently viewed this question with all the necessary reserve and circumspection. This circumstance prompted us to use the microscope in the resolution of a very important question, where all attempts up to the present have failed.

Let us admit that chemical reactions have determined certain stains as coming from dried blood; in the case where one would like to know to what species of vertebrate the blood belongs, one would not be able to decide at our present level of science. It is to this point that we have directed our attention; it is in this question that we have found a new opportunity for the use of the microscope, which, with the help of well-determined characteristics easy to grasp, can distinguish these different types of blood. We well know there will always be those who will rise against the use of the microscope, on the basis, in particular, of the varied illusions to which people unaccustomed to the use of this instrument are vulnerable; but the response to this question is quite simple; if physicians are not accustomed to this instrument, let them become so; their laziness or schedules cannot be an obstacle to the progress of science.

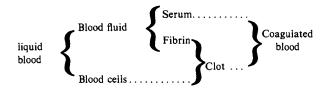
Chapter II. The Differences Which Exist between the Varied Types of Blood

Part I. Physical, chemical and microscopic properties of blood in the different classes of vertebrates

Everyone knows that blood having left the vessels coagulates and separates into two parts, one solid, called the *coagulum* or *clot*, the other fluid, which makes up the *serum*. The clot and serum consequently compose coagulated blood, or as one usually puts it, dead blood. When a more or less considerable portion of coagulated blood is left to itself, it will completely dry out, and there will remain only a fragile, brittle, solid crust of dark red, provided however, the amount of serum wasn't too great. In this case, putrefaction will settle in before dessication. This dry mass comprises all the

materials of blood, with the exception of water, which has evaporated; i.e., it is composed of the dried clot and the elements dissolved in serum, forming a dry residue when the water has evaporated. It is easily understood, then, that its size, its extension, etc., depends mainly on that of the clot which forms the most considerable part of the coagulated blood.

We say then that coagulated blood is composed of clot and serum. But circulating blood is quite far from presenting the same elements: examined under the microscope, in the transparent part of animals (tail of tadpole, tongue of frog, gills and fins of fish, intestines of young animals, etc.), it is seen to be composed, in the normal state, of corpuscles swimming in a reddish-yellow fluid. These corpuscles are usually called blood cells. What relationship then exists between the cells, the fluid suspending them (which we will call blood fluid), the clot and the serum? It has been known for a long time that the clot is composed in great part of fibrin, the same substance obtained as filaments in beating blood with stirrers. Microscopical observation, in addition, demonstrated that a clot contains whole blood cells, not separated from their envelope. Modern experiments, finally, which we have presented elswhere (Arch. Gen. Med., 1840, v. IX, p. 185), demonstrate that the fluid in which these cells swim contains dissolved fibrin. As soon as the blood has left the vessels, the fibrin dissolved in the blood coagulates, enclosing the cells in its mesh, and forms the clot. The blood fluid, deprived of its fibrin and cells, becomes serum. We can present this composition by the following table:



Let us now examine the principal properties either of coagulated, dried blood or of that still in circulation. It is understood that attention will be drawn only to those points relating to the research forming the substance of this paper.

A blood clot is red and soft; it is saturated with serum, which causes the softness. The color is from contained blood cells; the cells are colored red by the coloring matter of blood (hématosine), which dissolves when the clot is placed in water. The blood clot, composed, as we earlier pointed out, of fibrin and blood cells, consequently discolors when made to soften in water. The fibrin stubbornly retains a portion of blood cells; which is why it is necessary to macerate the clot in water, which it is necessary to replenish, until the liquid no longer colors. The finished product represents the fibrin entirely, white and uncolored, in soft, long masses, formed by intertwining filaments, similar to ribbons, whose volume is much less considerable than that of the clot which provided them. In this state, the fibrin is heavier than water, and sinks to the bottom. What we have just explained finds its entire application in the case of chemical examination of

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blood stains (Part II). Without further comment on the chemical properties of fibrin, we will only say that, in this state of coagulation, the fibrin is insoluble, in both hot and cold water, and is dissolved by caustic potassium, even when this base is very diluted. According to Berzelius, when fibrin is immersed in a caustic solution dilute enough to contact one's tongue without objection, it gradually transforms into a gel, as it does in concentrated acid, and finishes by completely occupying the solution. If one then encloses this in a closed vessel at a temperature of $50-60^\circ$, it dissolves bit by bit and thus produces a weakly yellow solution.

Dissolution of the red coloring material in blood is achieved by leaving the clot in water for a few hours, it being of little consequence whether it is soft or already dried. This dissolution plays a major role in medico-legal research on bloods. The chemical and physical properties of blood by treatment with different reagents have also been carefully studied. This liquid we are discussing, easily obtained by macerating the clot in water, is not only an aqueous solution of coloring matter, but contains, in addition, the elements of serum contained in the clot. The principal constituent of serum is albumin, to which it owes its most salient characteristics. In evaporating the serum, the albumin dries out, but is again soluble in cold water. When heating serum in a glass or porcelain container, at a temperature which gradually rises, it begins to lose its clearness at 65°, and at 75° coagulates into a mass the color of pearl, opaque, with translucent edges, insoluble in cold or boiling water. This appearance of coagulated albumin varies greatly according to the proportion of albumin and water. We saw earlier that albumin forms a solid, opaque mass; when the serum is diluted with a little water, the albumin will only form flakes. Further dilution of this liquid will cause coagulation to give it only a milky or opaline tint, becoming clearer as amount of water increases.

It is clear, then, what should happen when a blood stain is macerated in water. It dissolves the coloring matter of blood, which sinks in the form of reddish streaks to the bottom of the container. In addition it dissolves the dried albumin of serum; when heating this reddish solution, albumin forms flakes, or produces only an opaline tint in proportion to the amount of water used for the maceration. Finally, the insoluble part is fibrin; we have already pointed out its insolubility in hot and in cold water. These are the principal phenomena which take place when leaving portions of dried blood in water and which are of the greatest importance in medico-legal research of blood (Part II).

We are going to examine now the principal microscopic characteristics of blood, apart from any theoretical discussion, with the intention of rendering intelligible our studies on this subject (Part IV).

If a blood droplet is placed on a glass slide, and a very thin second slide is placed on the edge of the droplet, infiltration of the blood gives a transparent layer ready for observation. If it is blood of a mammal, one can see swimming in the fluid round, flat corpuscles, the diameter of which never surpasses

one hundredth of a millimeter; they are of a very pale red, almost yellow; they are the *blood cells*. Also evident is a second type of corpuscle, white, mammilated, with a diameter of at least a hundredth of a millimeter; we have called these elements *white fibrinous cells*, or simply *white blood cells*.

The blood cells have swollen borders on both sides, their center is depressed, which gives them the form of a very elongated "8" seen from in front, and when a considerable amount of water is added to the blood droplet, the blood cells, on examination after a certain time, are much more pale, almost entirely uncolored; their edges are scarcely visible. On the contrary, there are no changes in the white blood cells. This loss of color in the blood cells is more pronounced 564/ with greater amounts of water and with longer action on the cells. At the end of half an hour, there is no more trace of these corpuscles and one would think them entirely dissolved. However, on adding a little tincture of iodine, the uncolored cells become yellow, and are once again perceptible. The blood cells are not actually dissolved until after one or two days; but, we repeat, they are already so uncolored after a guarter or half an hour that they entirely disappear, because of their great transparency, and it requires long experience with a microscope to distinguish them in the midst of the serous fluid in which they swim.

We have spoken up to the present of the blood of man and mammals; but we've known for a long time that oviparous blood contains cells of an entirely different form; in addition, our observations have demonstrated that animals belonging to the camel family present blood cells similar to those encountered in oviparous blood. These blood cells are elliptical, instead of round as in mammals, and their large diameter almost always surpasses a hundredth of a millimeter. They are also flat and yellowish; but instead of presenting a central depression, they present on the contrary a central elevation, such that, seen from the side, they are bulging. This elevation comes from a central, oblong, granulated nucleus, which becomes more manifest as the cells remain for a longer time between the two slides. On dessication of a very fine layer of oviparous blood, one can see the central nucleus better in isolated cells. (For more detail, we refer the reader to our Anatomie Microscopique, 2nd series, 1st edition Paris 1839). In adding water to this type of blood, these cells also discolor, but their nuclei remain very distinct and do not at all disappear by the action of water.

Coagulated fibrin presents an amorphous mass, i.e., deprived of structure, white or gray, soft and elastic.

We have already previously pointed out that a clot consists of coagulated fibrin, which encloses blood cells. What does one see, then, if one macerates a portion of dried clot, for example, a blood stain, during a half hour or an entire hour? It is easily understood that when it's a case of mammal blood, one perceives only an amorphous mass, containing some white cells; the blood cells can no longer be distinguished. If, on the contrary, it is oviparous blood, all the nuclei remain distinctly visible. (cf. Part IV).

Part II. Chemical Procedures for distinguishing Blood from every other Substance

Every chemist concerned with expert medico-legal examinations now agrees that blood can be distinguished from every other substance, and they also agree on the specific means for reaching the results, since M. Orfila published his research on the subject (cf. *Jour. de Chimie Medic.*, Paris, 1827, vol. III, p. 367). The purpose of this paper is not to present those facts already known to science; we refer the reader who would like to acquaint himself with them to treatises on legal medicine. However, it would not be useless to say a few words to better understand our procedures which we are going to present later on (Part IV).

To find out the nature of a stain, it is macerated in cold distilled water, taking care that there is a certain distance between the stain and the bottom of the container. If it is a blood stain, red streaks are seen in no time at all going from top to bottom, and bit by bit, deposit on the bottom part of the liquid, coloring it red. At the same time, the stained parts thus treated by water discolor, and there remains in place of the stain a small greyish layer of wh⁺⁺ sh or reddish-white filaments. This layer, or the filaments, are formed by fibrin and the insoluble parts of blood cells; the reddish streaks, on the contrary, come from the red-coloring matter of blood, extracted from cells by maceration.

We must then clearly distinguish two essentially different parts: the liquid of maceration and the filaments. As for the liquid, it acquires a rose or reddish color when shaken in a glass tube; gradually heated to a temperature near boiling, it becomes cloudy, immediately changes color, and deposits flakes of coagulated albumin, or becomes only opaline. If the flakes are deposited, i.e., if a coagulum forms, it is greenishgray without the least trace of rose or red, and the supernatant liquid is uncolored or lightly colored in yellow-green; if the liquid is filtered and treated with potassium, it takes on a green tint, with reflected light, and a red tint with refracted light. If, on the contrary, it is not filtered, and treated with potassium while the coagulated albumin is suspended or deposited as flakes, the result is according to M. Orfila, about the same: the liquid acquires a reddish color by refraction, and greenish color when seen by reflection. There is not a coloring matter belonging to an animal substance which can produce this set of phenomena. As for the filaments, they are soft, a bit elastic, soluble in potassium; and the potassium solution, treated with chloride and a little hydrochloric acid, gives flakes of coagulated animal matter.

The chemist can always, with the help of these characteristics and of certain others, which we do not need to present here, distinguish blood stains from every other type of stain, for example, iron stains formed from lemon juice (iron citrate), rust stains, stains of substances which enjoy the property of coloring water a red or a rose (cochineal, Brasilwood, cartham, madder-wort, etc.). M. Raspail, far from sharing the opinion just aired, says: "it suffices to let a cloth sac filled with powdered madder-wort, slightly moistened with water,

sit for a few hours in chicken egg white; then to expose this mixture to a temperature of $25-30^{\circ}$ C, finally to dry it, to give it the appearance of a red stain similar to a blood stain". But M. Orfila fully refuted this opinion (*Jour. de Chimie Medic*, vol. IV); it would seem to us that the reaction with lime would be the only one to resolve the question.

There is then no doubt that legal medicine can distinguish blood stains from every other substance producing stains of analogous color.

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Part III. Examination of Methods Proposed for Distinguished different Types of Biood one from the other

At the reading of a work of M. Orfila at the Royal Academy of Medicine, which was reviewed by the Philomathic Society, at the meeting of July 14, 1827, M. Dulong remarked: "One of the most distinct characteristics of blood stains, even when they are quite old, is the form of cells seen under the microscope; it allows, in addition, distinguishing of the blood of different animal classes; dried mammalian blood cells present a white disc surrounded by a red circle, while in the blood of birds, the white disc is surrounded by an elliptic cell". (In this same meeting, M. Adolphe Brongniart said that beef blood had been able to be distinguished from human blood by microscope by M. Dumas in a medico-legal case; but M. Dumas hastened to point out the incorrectness of this assertion. It is certain M. Brongniart had confused two different facts). M. Orfila hastened to verify the opinion proffered by M. Dulong; but the conclusions he derived from his research, confirmed by Lebaillif, are not favorable toward the use of the microscope.

Indeed, the experiments of M. Orfila show: "1) that, even though blood contains a multitude of cells able to characterize it, it is sometimes impossible to realize the presence of these cells in dried blood on a slide, and even more on fabric, because the blood drop is too thick, contains only coloring material, or some other reason; 2) that, if it is true, in general, that mammal blood cells are circular, while those of the blood of birds and cold-blooded animals are elliptical, it is no less certain that in the study of blood detached from linen, elliptical and spherical cells in mammal blood, as well as triangular, square, etc. corpuscles, in the blood of birds can be seen, which probably results from an atom of dust or from the material of the fabric which united with the blood. It is easy to imagine that a cell which is spherical seen alone, presents another form when united with a foreign body" (Jour. de Chimie Medic., vol. III, p. 413; Paris 1827).

We see, then, that M. Orfila, in contrast to the opinion of M. Dulong, could not distinguish human from pigeon blood when removed from fabric, and "even sometimes that it was blood". We can understand how M. Orfila could arrive at these results when we examine the manner in which he conducted his research. Soon after "a portion of linen containing all the material of blood (of pigeon) had been left in a small amount of water until this was sufficiently colored, three drops of this liquid were deposited on a glass slide until dessication was complete". But what could this liquid contain? Assuredly hardly any blood corpuscles, for the greater part remained attached to the linen (Part IV), and the water dissolved only the coloring matter. At the time that M. Orfila was conducting his research, these properties of the cells had not yet been studied; we should not then be astonished to see this distinguished chemist looking for cells in this liquid and finding only irregular, elliptical, square, spherical, triangular, etc., corpuscles. These corpuscles are either molecules foreign to the constituents of blood, or some blood cells, detached from the fabric, not deformed by dessication, except many of them stuck together, in such a way as to form irregular corpuscles. This is the only way we can understand how M. Orfila would have found elliptical cells in human blood.

At other times, human blood dried on a sheet, dipped in water, and seen in the microscope before dessication, offered "a great number of small, transparent, spherical and ovoid corpuscles; on the other hand, it was difficult to find perfectly spherical corpuscles". Here again, it was scarcely blood cells which presented themselves, since they are almost all dissolved by water.

The result of this research is that the microscope is of no use in the examination of dried blood when only the dissolved portion of the stains is studied, on examination of either the dessicated or liquid state of the solution. As for the rest, we must remember here the influence of a few circumstances not well known at the time when the observations were made, and which would have hindered even the recognition of the presence of blood cells in the drop examined. For example, the drop examined should have been covered with a second glass slide to see it extended as a thin layer, and to see in this way all the suspended particles, whereas an uncovered blood drop presents for observation only the particles suspended on its surface. The alterations undergone by blood cells during a stay in water had not yet been well studied; it was thought, according to Hewson, that certain animals present corpuscles sometimes elliptical, sometimes circular. We presently know that every oviparous animal, coming out of its egg, always shows elliptical corpuscles, and that the circular form is due only to the effect of water on the cells.

It is evident after all we have just said that we had not arrived at a point of being able to distinguish the different types of bloods, and since these experiments have not been undertaken since that time, the result is that science has no microscopical method for distinguishing mammalian from oviparous blood. But before presenting our research on this subject, we will first say a word about a few chemical experiments undertaken with this end in view.

M. Barruel (Annals d'Hygiene, vol. I, P. 267) proposes the following method for the distinction of different kinds of blood. Blood is placed in a glass; one third or one half of its volume of sulfuric acid is added, and it is stirred with a glass rod; immediately a volatile aromatic element manifests itself, characteristic for each kind of blood. Immediately upon stirring, it is recommended to blow briskly into the glass, to

dispel the primary atmosphere, in which some sulfuric acid can be encountered. M. Barruel claims he can thus distinguish, by the odor alone, between the blood of man and of woman, and consequently the blood of the diverse species of animals. After the publication of these results, one must look to verify them, and many chemists repeated these attempts. Though several physicians have completely confirmed the results of M. Barruel, all agree, however, that the sense of smell is too fallacious, too uncertain, and most often too little developed in different people to dare apply M. Barruel's discovery to judicial assessment, however interesting it might be from the physiological point of view. On the other hand, to perform these experiments, a very considerable amount of blood is necessary, which is very rarely found in these assessments. It is true that M. Barruel asserts that even two weeks after the production of the stain, the species to which the blood belongs can be ascertained; but no one has verified this assertion. M. Morin of Rouen believed that he had discovered a great difference between the coloring matter of man and that of fish; but M. Lecanu showed the error of this opinion. M. Chevallier (Journ. de Chimie Méd) could find no chemical means for distinguishing blood stains from stains of bed bugs who has sucked blood and been crushed on fabric; the only difference is that stains of bed bugs, left by themselves for several months, ended by taking on an olive tint.

The result of all the preceding is then that legal medicine has no method, either microscopical or chemical, to distinguish among these different kinds of blood.

Part IV. Methods for distinguishing blood of man and mammals from oviparous blood

In a medico-legal investigation, once the nature of the stains has been determined and they are confirmed as coming from dried blood, there sometimes remains another question to be resolved. The accused might contend, admitting to the nature of the incriminating stains, that it is the blood of bird or fish found dried on his linen, knife, or hands. We have seen (Part II) that the forensic physician can, without hesitation or leaving the slightest doubt on this subject, determine the nature of the stains; but, from the facts we have previously presented (Part III), it is evident that the forensic physician has no method for distinguishing the blood of different species one from the other. We thought it necessary to do some research on this subject; we succeeded, not in resolving the entire question, but at least in distinguishing the blood of man and mammals from oviparous blood, i.e. blood of birds, fish, and reptiles. Here is the manner in which we proceed.

It is known that a blood stain macerated for a while in water discolors and that a small grayish layer or whitishgrey filaments of fibrin remain attached to the substance which bore the stains (Part I). It is this discolored fibrin which we examine; indeed, this alone can show the discolored cells, while the colored liquid coming from the maceration of the stain contains only the red coloring matter,

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Identification of Blood

dissolved albumin, and sometimes some detached blood cells. We are sure then that the microscopical examination of this liquid is of no use, and that it is necessary to submit the discolored fibrin to examination.

Here is the way to proceed to obtain the discolored portion of the stain adapted to microscopical examination; first, prepare a glass slide as for any other microscopical examination; place a drop of distilled water on the slide; then detach with any type of point whatever, most conveniently a cataract needle, a few particles of the stain; it is best to choose the edges of the stain, for here dried blood forms the thinnest layer, and consequently presents the most favorable conditions for microscopical examination. The particles detached in this way will have more or less the size of the eye of a needle; there will even be some which are smaller. It is always good to have at least four or five.

Once these particles of stain are procured, they must be transported into the drop of water placed on the slide. This is most easily done by slightly wetting the point serving to scratch the stain with some distilled water. All the particles will adhere to the point, which is then dipped into the drop of water on the slide, all the particles being made to drop by careful, light taps on the point. One must avoid rubbing the point against the slide, for this operation might alter the sharpness of the results. There will now be five or six particles, very small and very thin, floating freely in the drop of water: these are, so to speak, many microscopic blood stains. Now leave them for a while in the water to discolor them; one can easily see much less time is necessary to produce this discoloration than would be the case for a large stain. Indeed, after a quarter or half an hour, the particles are already discolored. To accelerate the dissolving of the coloring matter a bit, incline the slide in different directions. This will produce movements of the drop of water, which accelerates the discoloration.

When it is noted that the small particles have paled quite a bit, i.e., the coloring matter is dissolved, the examination proceeds in the following way: first, the amount of water in which the discolored particles are suspended is decreased by inclining the slide to pour off a part of the drop of water. A very thin second slide, one which usually serves as a cover for the object to be examined in microscopical observations, is placed with caution on the particles suspended in the water. Any considerable pressure must be carefully avoided. Those accustomed to microscopical observation will soon come to know the amount of water necessary to render the observation clear and distinct. There must not remain too much of the drop serving to dissolve the coloring matter, because the water will easily cover the second slide; nor must there remain too little, because the presence of air bubbles will render the particles too opaque. These are the precautions to take, by which one masters the technique in repeating it a few times.

We now have the discolored particles placed in a drop of water between two slides. The whole is placed on the stage of the microscope, and the particles submitted to exam-

ination. There is hardly need to add that the general rules, as in any microscopical observation, must be heeded, for example, as concerns light, etc. (On this subject, refer to our *Traité du Microscope*, Paris, 1839). In examining these particles, attention should be directed especially to their transparent edges: it is here the elements soon to be questioned can be distinguished most clearly. The central part is most often not sufficiently discolored: the examination is thus more difficult. Now here is what is observed in these discolored particles, which, as we know, are formed by fibrin and blood cells deprived of their coloring matter.

Particles of blood stains of mammals will present an amorphous layer, i.e., without any organization, in which here and there can be seen a few white cells. Of those blood cells which are completely discolored, there will appear no trace. When, on the contrary, the discolored particles belong to stains produced by oviparous blood, a very great number of oblong nuclei, crowded together, will be perceived in a very fine layer of coagulated fibrin, whereas the external contour of the cells will be no longer visible.

In this manner, one will have a very easy method for determining the species of blood producing the stain. But, the blood of man and that of mammals presenting cells of the same structure, it is easily understood that neither the blood of man and that of any other mammal, nor of one mammal from another can be differentiated by microscope. On the contrary, it will be very easy to establish if the stains in question belong to the blood of man or mammal, or to oviparous blood, i.e., fish, bird, or reptile. The blood of camels and all animals belonging to this family presents the same characteristics as oviparous blood; this is a result of observations we made in 1839, and which have been confirmed in a report made to the Academy of Sciences by MM. Milne-Edwards and Isid. Geoffroy-St.-Hilaire. This circumstance merits note, due to our possessions in Africa.

We reject the use of the microscope to distinguish the different species of blood of mammals: however, adherent fur can sometimes give very important information. It will thus not be very difficult to recognize fur of a rabbit, a steer, etc. or to differentiate them from hair. (See our Anatomie Microscopique, 1st series, 4th edition, Paris, 1840). The microscope might also determine, if necessary, whether the stain in question is actually composed of blood. In the case where chemical analysis has not decided this question, we reserve for another occasion the presentation of further details on this subject; however, we do not find it useless to add the following facts. Any mineral substance which can imitate blood stains will not discolor, and will show under microscopical examination a mixture of amorphous, red or opaque particles, without any trace of cells, which break under pressure. Fibrin, on the contrary, is white or grey, and elastic. Vegetable substances not presenting a grey layer like fibrin, the colored liquid obtained on their dissolution is amorphous, or presents a few vegetable parts which differ according to the plant examined. But we hasten to drop this subject, because chemistry has always previously determined the nature of the stain in these cases. When it is a matter of stains of bed bugs, one can discover parts of crushed bed bug by microscope in macerating the stain.

One might perhaps think it more advantageous to dip the entire stain in water, and then take a small portion of the greyish layer to submit to microscopical examination. But this opinion is erroneous: indeed, the entire stain macerated in water swells considerably, and the thin, transparent edges of these stains are thus lost to microscopical examination. Let us not here another circumstance which can sometimes be useful, but which we believe of no actual importance in the question occupying us. If the stain is not entirely discolored, the contours of the imperfectly colored blood cells are perceived in the fibrin layer; one can easily render these contours visible again by dipping the discolored layer in a weak tincture of iodine, or, even better, in a solution of sugar syrup (one in five parts distilled water) to which has been added a little tincture of iodine to color it slightly. The sugar solution does not alter the form of the cells; but we recommend these last procedures only to those already accustomed to the microscope because the thickness of the stain most often hinders distinct perception of the cell forms. Let us remark, finally, that the smallest stain can serve in a large number of microscopical examinations.

We have previously said that one will have "in this way a very easy method to determine the species of blood produc-

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ing the stain". However, it would be useful to present a few explanations in regard to this subject. When it is a matter of putting this research into practice, when one would like to make use of the results we have obtained in a medico-legal case, it must not be thought that just anyone can make these observations. A man is not a microscopist just because he owns a microscope: he must also be accustomed to it. In every case, we recommend to the physician the greatest reserve in the expression of his opinion. When he has confirmed the presence of nuclei, he can unhesitatingly pronounce the stain to have been produced by oviparous blood for two reasons: 1) because mammalian blood never presents a similar appearance; 2) because this judgment can only be in favor of the accused. But when the presence of nuclei cannot be determined, we believe it much wiser to announce a negative result, i.e., the physician would do better to claim that he could not confirm that the blood was oviparous; at least his opinion will not overwhelm the accused. On the other hand, he will avoid the error of wanting to make a judgment by a negative result, by the absence of nuclei, although from the scientific point of view the absence of nuclei is a characteristic as positive as their presence. We say in conclusion that, in practice, this research must only serve in favor of the accused, and that it appears to merit that much more attention from forensic physicians as it presents the sole scientific means for helping an innocent defendant.

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Memoir on the Examination by Microscope of Blood Stains on a Blue Cotton Smock in a Homicide Case*

Dr. Charles Robin

Professor of the Faculty of Medicine, etc.,

and

Dr. A. Salmon

Surgeon of the Hotel-Dieu of Chartres

/368 Examination of blood stains presents one of the most delicate problems in legal medicine. When limited to the use of chemical reagents in performing this examination, as happens in the greater part of assessments which come to attention in this context, the results are always incomplete or more or less approximate, which limits the operations to the use of only immediate principles, such as *albumin* and *fibrin*, and not the direct, constitutive elements of blood, i.e. white blood cells and red blood cells.

The use of the microscope, combined with that of reagents, offers then, in the research we are discussing, a security not found in any other procedure.

The details which we will present, on the occasion of an assessment with which we had been charged, will furnish very clear proof.

We will limit ourselves in this work to a description of the facts relating to the anatomic elements characterizing blood. But it is useful to point out that the guarantees of certainty and precision offered in the study of stains of various fluids by the means we have employed can be seen even more clearly with regard to animal and vegetable tissues. Their anatomic elements, invisible to the naked eye, but easily recognizable by microscope and less alterable than those of liquids, permit the differentiation of very small portions of these tissues at all ages of intra- and extra uterine life. Thus debris from envelopes of the embryo, the placenta, the decidual membrane, and blood clots from the matrix can easily be recognized; hair of man and fur from animals of varied ages, fatty tissue, nervous tissue, etc., can be clearly determined.

fatty tissue, nervous tissue, etc., can be clearly determined. It is known that it is precisely these cases where chemical methods are found to be ineffective.¹

§ I. Preliminary Questions

"... Given that this soiled smock, seized eight days after the crime, had not been washed since the crime, and that it preserved several blood stains by the very admission of the suspect, who recognized them.

Given that these blood stains are seen not only on the sleeves and on the front of the smock, but even on the back; that some of these stains, notably those on the sleeves and the lower part of the smock, appear to have been rubbed with either water or earth.

Given that Doiteau [the suspect] attempted to explain the stains by saying they came from blood spurting from a duck 37 killed in his presence . . .

Given that, even though this fact is inexact, and that the duck concerned had been killed out of the presence of the suspect, it is nonetheless important to determine if, *firstly*, the blood stains found on the smock are, or are not, blood stains from a duck, and if in any case the blood would have been able to spurt in quantity great enough to explain the numerous stains on the smock, on the sleeves, the front, and even the shoulders and back; if, *secondly*, this blood by its nature, color, adherence, form and the multiplicity of the droplets, is not rather the blood of a septuagenarian woman violently struck on the head with cutting and contusive instruments.

Given that in comparable circumstances the microscope is used today by science as a means of verification with the greatest success, etc.

In these circumstances, let us request, etc. . . .

Here are the questions concerning the smock the two doctors were asked to treat in their capacity as sworn experts:

1) Are the stains on the smock, and particularly the dark stains bordering on red and on yellow, of blood? (Do not limit your testimony to saying that they contain albuminous elements of blood, but say in no unequivocal terms if it is actually of blood, what is called blood.)

2) Apart from stains which appear to be blood stains to the naked eye by their color and form, are there not other stains on the smock of the same nature, but less colored as if someone had attempted to rub them off or to dilute them a little while after their formation by any type of rubbing or washing?

3) Are the blood stains in an amount large enough, and in places so multiple and diverse on the smock, that they could

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^{*} Translation of: "Mémoire Concernant l'Examen, a l'Aide du Microscope, de Taches de Sang sur une Blouse de Coton Bleu dans un Cas d'Assassinat". In Annales d'Hygiène Publique et de Médecine Légale 8 (2nd series): 368-397 (1857).

71 not be explained by splashes of blood of fowl slaughtered in in such a way as to submit each half to a comparable examthe presence of a man clothed in this smock and seated facing the fowl?

4) Are the elements of blood, of which the microscope permits recognition, elements of blood coming from a living duck who would struggle at the moment of having his throat cut?

5) Would not the elements of blood be, on the contrary, elements of blood belonging to the human species, belonging in particular (if science can go up to this point) to the septuagenarian woman violently struck on the head?

6) Finally, if these stains are of human blood, could not they have been produced, according to their placement on various parts of the smock, in the course of a homicide where a single man armed with a cleaver, a bill-hook, and notably a spade, had delivered fifteen blows to the head of his victim.

7) The appointed physician-experts will, in addition, present in their report the guarantee and certainty of the precision offered by the method of examination used by them in the execution of their assessment."

§ II. Examination of the blood stains by the naked eye and by magnifying glass.

To reply to the questions posed to us we proceeded as follows:

After having counted and measured the blood stains, we verified they were from $\frac{1}{4}$ of a millimeter to $\frac{3}{2}$ millimeters in width, all of them recognizable as such by their dull reddish-brown tint in daylight and shiny black by lamplight. They reflected this light with that peculiar glint, known to be one of the characteristics of blood observed under these conditions. But it is also known that this way of reflecting the

72 light of a candle, or of a lamp, is specific to stains of egg white, of gelatin, of gum and probably all stains of liquids rich in albuminous principles. However, the coloring in redbrown or blackish, together with the glint of light, present a specific characteristic for direction as to the means of investigation which might be used.

All these stains, examined by magnifying glass, demonstrated a small crust projecting slightly above the material of the fabric itself; each crust was brilliant under certain incidences of light, of a dull brown, on the contrary, when inclined otherwise. The thickness of the small crusts was so slight it was impossible to appreciate by the naked eye: about 1 to 2 tenths of a millimeter.

The small dimensions of the stains, and the thinness of the crusts forming them, soon indicated to us the impossibility of resorting to procedures based on examination of the coloring substance of blood and its albumin for determination of their nature.

But the existence of the small crust elevated above the material of the smock became one of the principal conditions permitting us to arrive at the certain determination of the fundamental parts of blood on each of the stains successively, despite their very small size. Stains of three and one half millimeters could even be halved by cutting the material

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ination by slightly different procedures.

§ III. Examination by microscope of stains on which had been determined by naked eye some of the characteristics implicating the stains to be of blood.

On a certain number of stains, of which we have just presented the external characteristics, after having halved the largest by cutting the material bearing it, we proceeded as follows to determine their nature, their intimate 373/ composition.

After division of the fabric supporting two of the previously indicated stains into the form of strips, they were steeped for six hours in pure water. For this preliminary operation, only the lower extremity of the strip bearing the stain was immersed in the liquid, such that two or three millimeters were left outside the water and applied to the wall of the capsule containing the liquid by the upper extremity of the strip. The fluid soon rose by capillarity up to the stain and gradually swelled the substance forming it.²

Once the swelling is complete, we removed the slightly swollen substance, by scraping the material a bit with a scalpel. We placed this substance in a drop of the same water placed first on the bottom slide of the microscope. After having dissociated the swollen substance in this drop of liquid with needles, it became a bit redder than in the dry state and we covered the preparation with one of the thin slides, or glass slides, used in every examination by microscope. This done, the preparation was placed under the objective of the microscope at a magnification of 514 actual diameters which demonstrated the following:

In the liquid of the preparation were seen more or less large fragments of the substance of the small crusts belonging to the stains. They were swollen by the liquid. These fragments were irregular, some greyish and others a bit colored by the previous particles. In addition, around the fragments, the liquid in which they were immersed was colored a red tint, similar to that given by the coloring substance of blood dissolved in liquid. The portion of the liquid thus colored formed a red zone, more or less wide, around each of the fragments of substance placed under the microscope.

Finally, either in the liquid of the preparation, or in the thickness of the fragments of the substance of the stains, were seen thin, microscopic filaments, twisted around each other, offering all the characteristics of filaments of cotton: all were uniformly of a slightly dark blue indigo, which contrasted with the red tint of the liquid of clear blood.

§ IV. Examination of the fibrin of blood in stains formed by this liquid

In adding some water under the microscope to the previously indicated fragments of the matter from the crusts, and even before this addition, we could determine very clearly that these fragments, swollen on contact with the liquid used, were formed principally of fibrin and secondarily by

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white blood cells.

The facts we are going to discuss below were also clearly evident:

1) Either in using pure water to swell the stains present on the last two strips of the four we had removed from the smock:

2) Or in scraping the small crust visible by magnifying glass on each stain and dropping this in small fragments, or powder, onto a drop of pure water placed on the bottom slide of the microscope.

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In proceeding thusly, water discolors the stains, or the matter removed by scraping; it renders the substance greyish, swelling it a bit; the water colors slightly red in accepting some of the coloring matter of red blood cells, whose uncolored elements it also dissolves after sufficiently prolonged action, without leaving any visible particle, such as nuclei or granulations.

After dissociation of the discolored fragments of the matter of the stains with needles, examination by microscope shows that they are formed principally by a transparent substance, scarcely greyish and finely granulated. In addition, the fragments of this matter placed under the microscope clearly demonstrated a fibrillary appearance of thin, rectilinear or delicately curved, interwoven filaments, some free and floating on the periphery of the fragments being examined.

After treatment with acetic acid, this fibrillary substance became extremely pale, gradually swelled, lost its characteristic fibrillary property and the fine granulations with which it had been strewn. It was thus observed to pass from the striated, finely granulated state specific to it, to the state of a homogeneous, transparent, gelatinous substance.

It is known that these attributes belong specifically to the fibrin of blood, and that taken on the whole, they result in an aspect entirely characteristic and constantly found by anatomists in this important principle of blood.

Thus the web of the small crusts or stains submitted for our examination was formed entirely of fibrin just as the web of a blood clot in blood-letting (which represents one of the crusts on a large scale) is formed entirely of fibrin, retaining in its thickness the two other characteristic solid parts of /376 blood, namely, the white blood cells and the red blood cells.

§ V. Examination of the characteristics of white blood cells retained in the fibrin of the cells.

On each of the fragments of the substance of the crusts placed under the microscope, formed by fibrin and cleared by water of the red blood cells it had carried along in coagulating, were recognized several white blood cells.

In the thickness of the fibrinous web just described were found transparent, greyish, round, finely granulated cells, of a width of 8 to 10 thousandths of a millimeter. In the center of several of them were also noted one or two small nuclei, greyish, irregularly spherical or oval, of a width of 3 to 4 thousandths of a millimeter. Such cells, in the middle of a web of fibrin, could come only from blood. In adding acetic

acid to these corpuscles, we demonstrated characteristics exclusive to such cells.

This acid, as its action gradually exerted itself, rendered the body of each cell transparent, slightly swelled it, and gave its contour a regular though paler aspect; at the same time, this reagent cleared away the fine granulations of each cell and showed their nuclei more clearly. These were soon presented in numbers of two or three, or even four, toward the center of each cell. They were disposed sometimes side by side, sometimes in triangle or horseshoc ar angements, or were superimposed. The reagent rendered the edges of these nuclei darker, easier to see, as can be noted on fresh blood comparably examined; it also rendered them a bit more irregular than the action of water alone.

The white blood cells, whose fundamental characteristics we identified, were sometimes isolated, scattered about in the fibrinous web of the stain, sometimes contiguous, united in groups of four or five or even in numbers two or three times greater side by side.

§ VI. Special examination of red blood cells retained in the fibrin of stains or adhering to filaments of the fabric of the smock.

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Knowing that solutions capable of preserving red blood cells intact are obtained by mixture or dissolution of various fluids and salts and that the cells regain their natural suppleness in these solutions after having been dessicated, we decided to make use of them, since experience had long since taught us their advantages.

These liquids were furnished to us by Mr. Bourgogne, manufacturer of microscope preparations, who, after many long attempts, managed to fulfill all the necessary conditions. This capable artist keeps the secret of the composition to himself, a secret we have not tried to uncover, each expert being easily able to procure this reagent for himself, in asking for it under the designation liquid 4_{a} .

Here is the procedure by which, with the help of this liquid, we easily found the red blood cells characteristic of blood in the stains submitted for our examination.

We worked with three stains, each of which furnished us with two preparations. One of the stains was a halfmillimeter, and the two others each a millimeter in width.

The first series of preparations was done by scraping the superficial crust of each stain and letting the scrapings fall into a drop of liquid. Covering this with a small slide, we let the small brownish fragments of the crust lie in the solution for twelve hours. There is no precaution to be taken during this maceration other than to protect the preparation from dust, for the liquid employed, being slightly hygrometric, will not evaporate.

At the end of this time, we saw that the small fragments of the scrapings of the crust immersed in the liquid were swollen, had become more transparent and redder than they had been at the beginning of the operation. They regained the characteristics of color, transparency, consistency and elasticity specific to the small groups which form during the

Identification of Blood

fresh human blood.

By the delicate maneuver of sliding the glass slides back and forth over each other, a maneuver to which one becomes accustomed in using the microscope, we succeeded without too much difficulty in detaching a rather large number of cells forming the group. We could then study the characteristics with as much ease as on fresh blood.

Each isolated blood cell had just about regained its circular, flat, biconcave form. Some still preserved a bit of the polygonal form which the reciprocal pressure in the accumulation had given them; others were concave on one side, as in the fresh state, but convex on the opposite side, as can be seen in blood cells placed in sodium or potassium sulfate solution. All were of 6 to 7 thousandths of a millimeter in width, rarely any bigger; this being the normal diameter of blood cells. All had regained their yellowish red tint specific to this species of blood element. Finally, in adding one or two drops of acetic acid to each preparation, the blood cells paled and gradually dissolved as in samples of fresh blood.

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It was possible in the previous examination to arrive more quickly at the swelling and dissociation of the red blood cells of the fragments of crust of a stain by adding a bit of water to the liquid used. Indeed, this liquid is prepared for preserving the elements of fresh blood; its action on those that have been dried is thus slow and even incomplete. But the addition of a small amount of water, slipped between the two glass slides of the microscope preparation, renders its action more prompt without removing any of the characteristic attributes of the isolated blood cells.

The second series of preparations, made with the three preceding stains, was done as follows. After scraping of the small crust on their surface, there remains beneath a small stain, paler, without projecting above the surface of the fabric, reproducing the size and form of the crust. Then, after cutting the surface of the fabric thus stained, with either scissors or a very sharp bistoury and dissociating the whole in the liquid used in the first series of preparations, the dissociated filaments were left immersed in it. In examining the cotton threads dissociated in the liquid after the same lapse of time, the characteristics of form and volume distinguishing the cotton filaments already pointed out above were evident. Also present was the blue indigo tint with which they were impregnated by the dye. But, in addition, we determined that many of them, in part or all of their length, were covered by a single layer of red blood cells or by small reddish agglomerations formed by blood cells of this type, accumulated and adhering to each other.

It was even easier here than in the first series of preparations to isolate red blood cells, to detach them from the surface of cotton filaments with the same maneuvers of pressure and sliding the glass slides. The characteristics of flattened, biconcave form, the volume, color and reactions on contact with acetic acid were all easily determined.

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On the greater part of the filaments, moreover, it was possible to identify red blood cells before they were detached.

accumulation under the microscope of red blood cells of They were perceived as having become slightly polygonal by reciprocal contact, but retaining their normal colors and dimensions, forming a layer on the surface of the cotton filaments.

> The blood cells presented themselves to the observer sometimes face on, sometimes sideways, at other times they were seen adhering to the cotton filament in half their length, while the other half being free, projected outward, showing its circular, flattened form.

> Thus, here again, no doubt remained: it was evidently red blood cells before us, an element found absolutely only in blood and it was the blood cells of mammalian blood and not of duck or any other species of bird.

> This conclusion was further verified by the reaction to the addition of excess water, or a small quantity of acetic acid, to the preparation placed under the microscope. The red blood cells adhering to the cotton filaments disappeared under our eves by the effect of these agents. Those which had been detached and the more or less irregular, voluminous accumulations formed by these blood cells existing here and there between the filaments, could be seen, as in the normal state, at first to swell, while paling at the same time, then gradually to fade by dissolution and soon to disappear altogether.

§ VII. Examination on the same blood stain: 1) of fibrin and white blood cells; 2) of red blood cells

With two other blood stains, one circular, of a width of one millimeter, the other oval, of the same width as the preceding, but two millimeters long, we proceeded as follows:

The small crust, red brown, served to make the first of a 381 series of two preparations. For this, it was placed for some time in pure water, after having been removed by scraping. It was left there until almost completely discolored, and was then dissociated with the needles designed for this purpose (see § III). Examined by microscope under a magnification of 514 diameters, all the characteristics of fibrin on one hand, and those of white blood cells on the other, as we have described above (see § IV and § V), could be recognized in the discolored fragments.

Having proceeded with these fragments of red-brown crust as for those of other stains, it was not astonishing to find the same blood cells retained between the thin fibrils of the web of fibrin.

Having placed some of the fragments of the red-brown crusts in the preservative liquid used above, they preserved their color and became slightly swollen. But by addition of excess water in one of the preparations and of a small quantity of acetic acid in the other (which was slipped between the two glass slides of the preparation according to standard procedure), these fragments were seen to swell a bit more, become pale, then completely dissolve before our eyes. Those treated with water left only a thin web of fibrin surrounded by a halo of liquid, weakly colored in yellowish red by the coloring matter of blood, which the water held in solution.

The small russet stain remaining beneath the crust removed from the surface of each of the two stains, and having the same form as them, provided another series of two preparations; but these preparations served specifically to see the red blood cells and in order not to destroy them to observe

/382 the fibrin and white blood cells, we proceeded otherwise than in the first. We used here preserving liquid 4_a mentioned in the preceding paragraph (see § VI). Having removed the surface of the stained fabric with a sharp bistoury, we dissociated it in this liquid and examined its filaments under the microscope, after leaving them in this same liquid for a few hours, either pure or diluted with about a tenth of its volume of water.

The red blood cells adhering to the surface of the cotton filaments were thus rendered perceptible (see § VI, the description of the second series of preparations, p. 379).

By the same procedure of sliding the glass slides, we could isolate the red blood cells from the filaments, so as to determine their characteristics of form, again generally circular and flat, of biconcave disposition, of the volume, the color and reactions already indicated. It is important to note that the central spot of the red blood cells of man, indicating the central depression of the two faces, in a word their biconcave disposition, is less pronounced in blood cells which have been dried and then softened than in fresh blood cells. In a word, these anatomic elements, after softening and isolation in blood stains, do not regain a biconcave form as pronounced as they show in the fresh state. But this property does not hinder their easy recognition, when one has already observed human blood in its various conditions.

It is thus evident that, with a single stain, the existence or absence of the three most characteristic constitutive elements of blood can be determined by microscope. The superficial crust should serve to determine the characteristics of fibrin and white blood cells; whereas we maintain that the subjacent threads of fabric, between which the blood serum has infiltrated, carrying with it red blood cells, /383 should be reserved specifically for demonstrating the exis-

tence of red blood cells.

§ VIII. Examination of the characteristics of blood cells in stains formed by the blood of a duck

In response to the questions posed to us by the rogatory commission of the examining magistrate, we let the blood flowing from the carotid arteries of a duck we had decapitated fall in a shower of droplets onto a blue cotton smock. Having left these stains in a dry place for two weeks at the normal temperature for this month of January, 1857, we proceeded with our examination, adopting exactly the same methods as for the examination of the blood stains on the smock of the suspect Doiteau.

After treatment of the small detached crusts of the blood stains of duck with the preserving liquid $(4_a, \text{ see } \S \text{ VI})$, a certain number of oval-flat blood cells could be isolated, at least twice as large as those of man, and bearing in their center a small, oval, elongated nucleus, not less characteristic of the blood cells of fowl than the elongated, oval form of the cells themselves. This small nucleus soon became very

evident, with clear, well delineated edges, under the influence of excess water and of acetic acid which consistently produces the effect of dissolving the reddish body of the cell, and leaving the greyish nucleus intact without any specific color.

After treatment of the small crusts detached from the blood stains from a duck with pure water, they gradually discolored, becoming greyish. For a relatively long time, they remained surrounded by a layer or halo of liquid colored in pale blood-red by the coloring matter of the blood cells, extracted by the water from the mass of accumulations of blood cells submitted to experimentation. Once the discoloration is practically complete, it was determined that no 3t evident fibrinous web remained in place of each fragment, as was the case in the blood stains suspected of having come from the body of a woman.

There remained only a considerable number of oval, greyish nuclei, without any coloring specific for blood cells of duck. These nuclei were 5 to 6 thousandths of a millimeter long, half of this diameter wide and thick. They were all very close to each other, the greater part remaining agglutinated by a small amount of uncolored substance, in which the fibrillary aspect specific to fibrin could be confirmed only with difficulty. Acetic acid soon rendered the nuclei darker, their edges blacker; at the same time tightening them and rendering them a bit less regular, the usual action of this reagent on fresh blood cells of birds.

It was impossible to identify white blood cells in this mass of nuclei remaining after the action of water and of acetic acid on these fragments of crust removed from the blood stains of duck.

Thus: 1) the oval form and the doubled volume of the blood cells here examined compared to those encountered on the stains of the smock of the indicted; 2) the absence of nuclei in the latter, the usual case in man, and the presence of oval nuclei in the blood cells of all birds, does not permit us to acknowledge that the blood stains on the smock submitted for our examination are formed from the blood of duck or any other fowl. These characteristics permit the easy identification of the nature of the blood of man in one case and of the blood of bird in the other, with no possible confusion, since the flat, circular form with the absence of nuclei is constant in blood cells of man after birth, whereas the flat oval form with a central oval nucleus is constant for every 3ξ blood cell in birds.

In addition, the differences derived from the almost complete absence of the fibrillary fibrinous web in the blood of birds, as compared with that of man, in which this web is abundant; the small number or absence of white blood cells in the blood of the first as compared to their notable quantity in the fibrillary web derived from human blood stains: here are distinctive characters which, although of second order, have a value not to be neglected by the expert.

It is useless insisting on an understanding that these observations should be considered as the most delicate of those which a legal physician might be called on to make, in that

all these characteristics are obvious only after repeated observations of blood of diverse animals in diverse conditions, and to persons accustomed to judging the value and precision of the nature of these facts.

§ IX. Examination of other stains of the smock, less colored, presumed to be of the same nature of the first, i.e., presumed formed by blood and attempted to be rubbed out or diluted a little while after their formation by some type of rubbing or washing.

We examined microscopically either the powder, or the filaments of the fabric of the smock, detached from the large stains of the sleeves, the front and the back of the smock, stains of a reddish-brown, almost the color of rust, or analogous to those which might be formed by blood which was wiped, half-washed or rubbed with earth.

It was immediately recognizable that the isolated, free microscopic fragments, as well as those still adhering to the filaments of blue cotton, were composed of small, irregular, log polyhedric, angular grains of multiple facets, unstructured in their reciprocal disposition. Some of these grains were without any specific coloring, greyish or uncolored in the center, more or less brilliant, with thick, blackish contours.

Their diameter varied from 5 to 70 thousandths of a millimeter and more. Water was without effect on them; acetic acid, added to the preparation, scarcely attacked them, releasing a few bubbles of gas from their substance. Only hydrochloric acid dissolved them rather rapidly with release of a certain quantity of gas.

Others of these irregular grains, a bit less in number, presented the same irregularities of form, but were of a rather brilliant red-brown tint, which can be noted by microscope in various oxides and especially iron carbonates. These irregular, red-brown fragments had a diameter varying between 4 and 35 thousandths of a millimeter. Water had no effect on them, acetic acid, added to the preparation had an effect, but very little, only at the end of several hours. Such that, in this report, no more than in the preceding, there is nothing comparable to the fragments from the crusts of blood stains. These irregular grains were, on the contrary, quite rapidly attacked by hydrochloric acid in the same way and in the same time as the uncolored grains mentioned above with which they were mixed.

The characteristics just mentioned being those that the microscope exhibits with the majority of terrestrial powders, and having none of those which this instrument, with the aid of chemical reagents, exhibits in blood, it was necessary to investigate their nature and their composition with the help of the appropriate reagents furnished by science.

To achieve this, we proceeded as follows:

First, thirteen stains of a red brown were removed with a sharp bistoury. The irregular ones were spread out; the others were small, round, 1 to 3 millimeters wide, superimposed on the large, less-colored, russet-like stains on the lower part of the right sleeve. We didn't touch the left sleeve.

The same operation was performed on four small stains, 1

to 4 millimeters wide, of a brown-red, on the back of the smock, which were not used in our previous operations, and which were also evidently superimposed on the large, lesscolored, russet-like stains of this part of the smock. We left the front of the smock intact. The matter thus removed, and submitted to examination by microscope and with the reagents already employed, showed successively the characteristics of fibrin and white blood cells retained in its thickness, and red blood cells, a constitution similar to that of stains that the same examination indicated as being formed from blood. The paler stain remaining after removal of the small, obviously red crusts previously indicated, while having the form and dimensions of the others, no longer showed blood cells adhering to the blue cotton threads, as had been seen on the analogous stains taken from the cleaner portions of the smock. We found here only fragments or irregular grains of powder, some greyish, without any specific color, the others of a deep red, such as those described in the parts of the large, less-colored, russet-like stains bearing none of the small stains of a dark red-brown.

This done, the large, *russet-like stains less colored* than the small one, as many of them on the lower part of the right sleeve as on the back of the smock, were carefully scraped with a scalpel. A large porcelain capsule received the powder falling from them. This was then collected in the capsule by washing the capsule with hot distilled water. The murky, clouded water thus obtained was left to cool until the following day. As a result it separated into three parts:

1) The *first part*, floating on the surface of the liquid in the tube, was flocculent and blue. Submitted to examination by microscope, it was shown in be composed of filaments of cotton tinted in blue, accompanied by irregular particles presenting the aspect of the grains of the powder described at the beginning of this section and not being affected by water. Moverover, these particles were present in such a small amount that it was useless, in the presence of the facts which follow, to do a special analysis. We then rid ourselves of this flocculent magma of cotton filaments tinted in blue.

2) Separated from the liquid, and deposited on the bottom of the tube, was a finely granulated powder forming a thick layer of 8 millimeters in our tube, which was 15 millimeters wide. Decantation isolated the powder from the water from which it had separated by gradually depositing, because of its more considerable specific gravity. Examination by microscope showed it to be formed entirely of irregular corpuscles, some grey, without any specific color, others of a red brown of oxide or iron carbonate, such as those discussed at the beginning of this section. A few, adhered to the microscopic filaments of cotton, which had been carried along to the bottom of the water in depositing.

This powdery deposit was placed aside for submission to an analysis, the details of which will be developed later on.

3) Finally, the liquid interposed between the blue, flocculent magma of cotton filaments floating on the surface and the powder deposit described earlier was examined separately after decantation. This liquid was uncolored, but of

a cloudy tint of a bluish grey. The microscope soon showed ^{/389} that this cloudiness was due to very short, broken, cotton filaments suspended in the water and also tinted in blue. This

liquid was submitted to boiling, which caused neither any new cloudiness, nor coagulation, nor clarification. The liquid, not having changed its aspects, was filtered and then became perfectly clear. Heated again, it remained as such. This liquid was also put aside, to undergo a special analysis, the results of which will be presented in the following section.

From this preliminary examination, we have already been led to several conclusions which it is important to point out at this moment for they guided us in the use of the methods of analysis remaining to be presented, and which served only to confirm these conclusions.

1) Introduction of the powder derived from the large, russet-like stains into water did not change the aspect of this powder nor color the distilled water in red or wine rose. The examination by microscope, which hadn't shown any elements of blood in these powdered particles, was thereby confirmed; for the powder derived in infinitely less quantity from the scraping of blood stains was enough to noticeably color an equal amount of distilled water.

2) The absence of coagulation in the liquid submitted to 100° temperature, before and after filtration, showed that it held no albuminous substances in solution.

Consequently, this chemical examination already demontrated, as had the microscope, that it was a matter of mineral powders only, of an origin outside the human body, and not of blood stains which were half-washed or submitted

/390 to rubbing or incomplete wiping.

§ X. Concise exposé on the chemical analysis of powder coming from the stains, which had been considered by their russet-like tint as possibly formed by blood which had been washed or wiped.

The elements which distilled water had removed from the powder of the stains were first determined.

For this, it was divided into three parts in as many different tubes.

Silver nitrate gave in the first an appreciable white, flocculent precipitate which dissolved in ammonia, indicating the presence of a small amount of soluble chlorides.

Barium chloride produced in the second tube a very abundant white precipitate, which did not dissolve after acidification of the liquid with a little sulfuric acid. This reaction showed there existed an appreciable quantity of soluble sulfates.

In the third portion of the liquid, a very light blue color was obtained by addition of potassium cyanide, indicating traces of iron peroxide salts. This tint became a bit dark only after evaporation by heat reduced by half the small quantity of liquid tested.

Water, acidified by hydrochloric acid, was poured on the powdered deposit already discussed, and set aside for a future analysis. The whole mass was seen to dissolve in the

space of about an hour, producing the release of small bubbles of gas. The small amount of matter precluded its collection; but everything pointed to the conclusion of carbonates decomposed by hydrochloric acid, displacing carbonic acid.

The resulting solution offered a very light bluish tint; the liquid thus obtained was still appreciably acidic for it obviously reddened litmus paper. It was divided into three equal portions in separate tubes.

In the first, addition of a small amount of barium chloride gave no precipitate; but the addition of ammonia to the point of neutralization produced an abundant white precipitate of barium phosphate. A white flocculent precipitate was also produced when excess lead acetate was added to another portion of this liquid. These characteristics indicated a certain amount of phosphoric acid, most of which had combined with lime, to form irregular microscopic grains of mineral powder observed by microscope and deposited in distilled water. The following reactions attempt to prove this:

In the second portion of liquid, excess potassium oxalate was added, which immediately produced an abundant granular precipitate, rapidly collecting at the bottom of the tube, and formed by oxalate of lime.

Finally, in the third portion of the liquid, addition of yellow potassium ferrocyanide developed a very pronounced Prussian blue color.

As in examination by microscope, these reactions demonstrated 1) that the powdered matter derived from the russetlike stains, less colored than those presenting red blood cells and fibrin, were not composed of elements of blood nor by other substance of animal origin.

2) that this powder was composed of cotton filaments in small quantity, but principally by mineral substances, such as are generally found in the greater part of terrestrial powders.

3) that these latter substances were irregular grains, some greyish, with no specific color, formed mainly by phosphate 3 and lime carbonate, with traces of soluble sulfates and chlorides, and probably also lime sulfate.

4) that the remaining irregular grains, less abundant than the preceding, but giving the powder its tint of russet-like grey, were undoubtedly composed of iron oxide and carbonate, whose presence the reagents disclosed in a quantity much more considerable than that contained in any animal substances.

5) that consequently, the less-colored stains, apparently rubbed or diluted by some kind of rubbing or washing, were not produced by spread-out blood, but by powder or mud, soiling the smock of the accused, before perpetration of the crime.

In addition, this conclusion is confirmed by the fact that on the stains identified as being formed by mud, the existence of superimposed blood stains could be demonstrated, presenting the same characteristics as those found on stains scattered on the surface of the unsoiled parts of Doiteau's smock.

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\S XI. Response to the questions posed by the examining magistrate[†]

Firstly, recognition that the greater, russet-like stains, the lightest in color, are not composed of blood, but by terrestrial matter mixed with particles of iron oxide and carbonate forming rust, permits the response:

Yes, blood could have spurted from the carotid arteries of a decapitated duck in great enough quantity to form or explain the formation of the numerous but small stains actually composed of blood on the sleeves, the front, and even the shoulder and back of the smock.

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Secondly, and a fortiori, these multiple droplets, whatever their form, could come from the arteries of the soft tissue of the head of a sixty-eight-year old woman, when these arteries are torn or cut by violent blows delivered to the head with cutting and contusing instruments: and this is strengthened all the more since examination of the indicated blood stains peremptorily demonstrated that, by their constitutive elements, their nature was that of drops of human blood, having none of the characters found in the blood of ducks.

The undersigned sworn experts can then resolve the questions posed in regard to this smock as follows:

1) Yes, the dark stains of the smock, bordering on red brown, are of blood. They are formed by blood, without the water which renders it fluid, because this water was released by evaporation after the blood left the vessels.

Indeed, only in blood are found the red blood cells we succeeded in isolating from these stains; only in blood are fibrin and white blood cells, which were identified in the thickness of the web it formed, and the red blood cells which were isolated grouped together.

Only the microscope could have decided this question, because these stains were too small for it to be possible to demonstrate the existence of blood albumin. Besides, it is known that albumin or analogous albuminous principles with the characteristics found on large blood stains can be encountered not only in a large number of animal but also in colored and uncolored plant sap. On the contrary, only blood offers together, simultaneously, fibrin, flat, circular red blood cells without nuclei, and spherical white blood cells 1/394 furnished with one to three nuclei after the action of water or of acetic acid.

2) No, apart from the stains which appear to the naked eye to be blood stains by their form and color, there are no other stains on the smock of the same nature, but less colored, which would have been incompletely effaced or diluted a little while after their formation by some sort of rubbing or washing.

Indeed, examination of the substance of these large stains, less colored, russet-like or bordering on yellow, demonstrated they contained none of the elements of blood. This same examination by microscope, completed by chemical analysis of the substance retrieved from the indicated stains by scraping, demonstrated they were formed by irregular grains of a mineral nature. Some were composed of insoluble calcium salts, such as phosphates and primarily, carbonates, with traces of soluble chlorides and sulfates. The rest, a deep red brown under the microscope, were composed of iron oxide and carbonate, elements composing rust, often encountered in mud or other soiling matter. But on these mud stains, or at least russet-like stains, were found superimposed blood stains, presenting all the characteristics to the naked eye and by microscope indicated above found in the stains of the aspect scattered on the unsoiled parts of the smock.

3) The stains which were actually of blood, without its water, were not in great enough quantity to be able to conclude that splashes of blood from a fowl could not have produced them. Blood spurting from the carotid arteries of a decapitated fowl, could, if this fowl were not on the ground, rise high enough so that some one facing the person killing the fowl could receive blood in places as multiple and diverse as those on the smock submitted to us.

4) But the elements of blood composing these stains 39 are not those of the blood of duck. These elements have, on the contrary, all the characteristics of elements composing the blood of man. They do not have the flat, oval form, nor the volume, nor the central, ovoid nucleus found in red blood cells characteristic of fresh or dried blood of ducks and other fowl.

5) The elements of blood forming the stains of the smock are elements of blood belonging to the human species. Fibrin is found, with its fibrillary aspect, its reactions on contact with acetic acid, etc. White blood cells are found with the volume, form, granulations, nuclei and chemical reactions found in the white blood cells of the blood of man. Red blood cells are found with the volume, the flat, circular, biconcave form, the rosy yellow color specific to those of man viewed by their transparency in the microscope and dissolving like them in water and acetic acid without leaving a trace of nuclei.

But it is impossible to say more with our present level of knowledge; it is impossible to determine with this blood the sex or age of the individual from whom it originated.

6) Yes. Finally, these blood stains, for they are stains of human blood deprived of its water after dessication, scattered and small as they are, could manifestly have been produced, there, where they are, present on various parts of the smock, by the spattering of blood from veins opened by a violent blow. Or, better, from the spurt which arteries give off before death during a murder where one man, armed with a cleaver, a bill-hook or a spade, would have delivered fifteen blows, or even less, to the head of his victim.

7) The undersigned medical experts, in presenting their methods in their report, demonstrate, by the detail into which they have gone, that the means of verification employed by 39 them in the execution of their assessment offers safeguards, a security and a precision superior to means employed up to the present. Indeed, the microscope alone permits seeing, not the albuminous or ferruginous elements of blood, but its very composing elements, which are most characteristic, permit-

[†] See § I, pp. 369-371.

Identification of Blood

ting the claim that a liquid is blood and not any other liquid. Only the microscope permits the determination with a single stain, be it only a millimeter or more in diameter, of the existence and all the attributes of the three solid parts, the most characteristic, of blood, namely: fibrin, red blood cells and white blood cells.

Finally, the safeguards and security of precision of the procedures they employed are emphasized with the very strong evidence of the following fact. This fact is that only examination by microscope can determine if the indicated stains are formed by blood of duck or of man, because, as we have just pointed out, it shows the very elements which float in blood, giving it its color and its other characteristics. These elements, then, differ between man and birds, reptiles and fish by their form, volume and intimate structure, of which only the microscope permits the determination. And it shows them, wherever these elements might have been deposited, unless putrefaction has set in.

Besides its application with an equal precision on small stains, even on one small stain, and on large stains, this method offers still other advantages over those generally employed up to the present. Indeed, these latter are based only on examination of the coloring substance of blood, on

that of the iron it contains and on that of albuminous matters of blood soluble in water. These elements are identical, with no possible differentiation, with regard to color, reactions, etc., in the blood of man, birds and other red-blooded animals; such that the questions posed to us, relative to the nature of the stains, concerning the determination as to whether they are formed by the blood of man or of duck, remained absolutely insoluble without the use of the method of verification we had adopted, either as a method in itself, or in using the older procedures as a simple adjuvant to the microscope.

References

- Ch. Robin: Sur la distinction, à l'aide du microscope, de la matière cerebrale, de l'albumine, du fromage et du jaune d'oeuf; observations publiées à la suite d'un mémoire de M. Orfila, intitulé: Recherches médico-légales sur la matière cérébrale desséchée, tenteés, à l'occasion de l'assassinat de Louvet, par Gontier (*Ann. d'Hyg. et de méd. lég.*, v. 44, p. 190, avec une planche gravée).
- This procedure must also be followed when examining stains of semen, of vaginal, nasal or urethral mucus and of meconium or fecal matter. See. Ch. Robin and A. Tardieu, Mémoire sur l'examen microscopique des taches formées par le méconium et l'enduit foetal, vol. VII of the 2nd series of this collection, 1857.

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Memoir on the Medico-legal Comparison of Stains of Menstrual Blood and Other Types of Blood Stains* Charles Robin

Professor of the Faculty of Medicine of Paris, etc.

⁴²¹ § I. Preliminary observations on the question to be resolved in this work. In the preceding memoir, we made known under what circumstances we were committed by Mr. Edward Choppin, examining magistrate of the borough of Chartres (Eure-et-Loir), to compare stains of mentrual blood with other types of blood stains.

It was a question here of making a positive determination of whether blood stains indicated by the accused as coming from menstrual blood were actually of this origin; whether they were not formed rather by human blood coming from wounds produced by telling blows having death in mind; or finally, whether there were not stains of both types of blood simultaneously on the same sheets and shirts.

It has been impossible up to now to resolve questions of this kind. Procedures available to legal medicine for the differentiation of blood stains of a menstrual period from other types of blood stains are limited to but one, that of Barruel. This procedure consists of pouring concentrated sulfuric acid on the blood, and its origin is recognized by the odor *sui generis* which is released, an odor different between one animal and another, different also between the blood of man and the blood of woman. But this procedure has been rightly considered, by its author and by forensic physicians, as liable to give only presumptions on the origin of blood stains and not proof leading to legitimate conclusions. We could not find any work mentioning its use in a legal assessment. We thus believe it has never served to resolve a question of the type for which we had to find the solution.

As we had succeeded experimentally in determining in a precise manner the characters distinguishing the types of stains we have been discussing from each other, we considered it useful to publish the results of our research. Our experiments were performed as a result of the questions which had been posed to us. As these are summarized in a way by the description of the procedures which we definitively adopted for the study of the evidence submitted for our examination by the examining magistrate of Chartres, we will limit our presentation to the steps we followed and the results we obtained. We will, however, do a preliminary presentation of peculiarities relative to menstrual blood under normal conditions with which it is necessary to

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be familiar to understand the value of our medico-legal experiments. As is known, this matter has already been the subject of very precise observations by microscope by Pouchet.¹

§ II. Examination of the anatomic constitution of menstrual blood. At the beginning of a menstrual period, when the mucus which flows from the vulva or just moistens the labia begins to stain the linen in reddish brown, it is found by microscope to be constituted as follows: In a more or less viscous, finely granulated liquid are seen some not very regular, prismatic epithelial cells and some nuclei of nuclear epithelium resembling that of the uterus, undoubtedly coming from the mucus of the uterine body and cervix. But especially found here is cuboidal epithelium of the vagina, of finely, uniformly granulated cells, the greater part of which are furnished with nuclei, sometimes nucleoli, and which we don't have to describe here, as they should be known in all their conditions to anyone working in medico-legal research. Leukocytes (pus or mucus corpuscles) are also seen in greater or lesser quantity, according to the subject, especially numerous in women in whom catamenial congestion determines the appearance of *leukorrhea* or purulent mucus. Finally, a certain quantity of red blood cells are found, to which the reddish hue of the liquid is due.

After twelve to twenty-four hours of this weak discharge, which may or may not be followed by an interruption which can last an entire day, the uterine hemorrhage gains its full intensity.

The liquid flowing from the vulva, collected from the vulva with a curette or spatula, is much more fluid than before, because of the amount of blood serum, mixed in with the mucus. However, the same elements we have been discussing are always found here; the red blood cells nevertheless outnumber the epithelial cells and leukocytes. The delicate fibrillary web formed by coagulated fibrin is not produced in the blood, though it is observed in drops of blood, a bit voluminous, drawn from the finger for examination by microscope, a fact resulting from the influence of mucus on blood plasma and particularly on fibrin. It is not uncommon, however, to see red blood cells united in stacks between the epithelial cells or the bits of epithelial cells which accompany them, as they do in blood drawn from a finger or in a bloodletting. However, a certain number of molecular granulations are always found in the muco-serous liquid in which

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^{*} Translation of: "Mémoire sur la Comparaison Médico-légale des Taches de Sang Menstruel et des Autres Espèces de Taches de Sang." In Annales d'Hygiène Publique et de Médecine Légale 10 (2nd series): 421-434 (1858).

¹ Pouchet: Theorie positive de l'ovulation spontanee. Paris, 1847, in-8, p. 241-244 et atlas, pl. XII et XIII.

these elements are floating, such as those encountered in most mucus, that of the uterus in particular, and which is lacking in blood serum.

After a duration of two or three days this blood flow becomes thicker, regains a mucus consistency, and at the same time loses the pure red color it presented. It takes on a reddish or reddish-brown hue. The nature, consistency and viscosity of the liquid holding the anatomic elements in suspension, becomes apparent. The molecular granulations are once again abundant, as well as mucus corpuscles and the different varieties of epithelium, as much nuclear and prismatic as cuboidal; the latter, however, are always more numerous. These cuboidal cells, as those of the vagina in any other condition, are for the most part provided with nuclei; but a few of them are lacking in nuclei. Many are wrinkled, and sometimes others are grouped together in pieces or lamellae of overlapping cells, sometimes large enough to be

/425 perceptible to the naked eye. In bloody mucus flowing at the end of menstruation, it is not uncommon to also find flakes or filaments or homogeneous or striated uterine mucus, including within their thickness ovoid nuclear epithelia similar to those described above. At this period, the red blood cells no longer gather in stacks and are small in number. In all stages of menstruation, moreover, the number of mucus cells varies widely from one subject to another and some show almost none at all.

§ III. Examination by naked eye of the stains whose nature we were to determine. The shirt under examination is an old women's shirt, patched at the right shoulder, and mended in several places; it is marked in front on the bosom with the letter "M" in partly discolored blue thread.

Numerous blood stains exist on this shirt which is reddish in front and in back, inside and outside, from the waistline to the lower border. However, in considering attentively the disposition of these stains, it soon appears that, despite their number, almost all of them have a common origin in the interior of the fabric of the article of clothing, in that part of the shirt corresponding to the position of the genital organs of a woman.

Whence, three types of stains:

Stains of the first type. Thick stains starching the entire thickness of the shirt, with a possibility of coming as much from the outside of the garment as from the inside and of a very deep red color. These stains are found especially on the back of the shirt, where they occupy an extremely considerable space of about 18 centimeters. In this part, the stains are not isolated, and despite the vertical pleats of the shirt where the color is a bit less dark, they evidently form one bloody sheet, in the middle of which some dried clots are encountered towards the inside. Stains of this type, but a bit

/426 less extensive, can be noted also: 1) in the front of the shirt toward the middle; 2) on the lower border in front; 3) on the lower border in back, but these last stains, instead of presenting a bloody sheet extending about the same distance in all directions, are, on the contrary, very long with no width, as if they resulted from imbibition of a thin trickle of

blood.

Stains of the second type. Thick stains, evidently produced by bloody imbibition from the inside to the outside, also starching the material of the garment, and of a deep red color. These stains exist only on the inside of the shirt. They are more numerous in back than in front. They occupy, on the limit of the preceding stains in back, a rather considerable space, but with a lesser surface, and present a large number of gaps. Many of those stains have an irregularly rounded form, and seem to have been produced by dried drops of blood.

Stains of the third type. Stains not starching the material of the shirt, existing only on the inside of the shirt, without imbibition of blood, of a somewhat deep pale-rose color. Just about all these stains are very long from top to bottom, and measure scarcely 2 to 3 centimeters in width. They are individual, separated from one another by not very large vertical spaces in which the fabric of the shirt takes on a soiled yellow color. They occupy especially the inside back of the shirt, a part of the lower border on the back, and in front they merge with some yellowish stains apparently due partly to imbibition by the material of urine or vaginal mucus.

On the bed sheet were found not very numerous, rounded blood stains, successively placed one after the other similar to those described above under the designation of stains of 4: the first type. It is useless then to reproduce a detailed description here as we have already written it.

§ IV. Examination by microscope of blood stains claimed to have been formed by menstrual blood. To determine the nature of stains we had to study here, we proceeded in the same manner as if it had been a matter of pure blood stains. We won't review the methods employed for this, for they are absolutely the same as we had presented in a work previous to this one. (Ch. Robin and Salmon, Mémoire concernant l'examen de taches de sang a l'aide du microscope, Annales d'hygiene et de medicine legale, Paris, 1857, vol. VII, §§ III and VI; and in Briand, Chaude and Gaultier de Claubry, Manuel complet de médecine légale, Paris, 1856, 6th edition, pp. 705, 707, 805 and 807.)

After the strips of fabric bearing the stains, which were softened by the procedure just mentioned, were scraped with a scalpel and placed under the microscope, numerous filaments of hemp were perceived. These were surrounded by small masses of red hue, on the edges of which red blood cells adhering to each other, a bit deformed, but still flat, could be recognized. Some were isolated and showed their biconcave form or were concave on one side and convex on the other. These blood cells had become pale, had swollen on contact with water and dissolved after addition of acetic acid. In similar strips softened in pure water, microscopic filaments of hemp were surrounded here and there by small red masses or magmae, finely granulated, in which the fibrillary disposition of fibrin, which are shown by clots of stains of pure blood treated with pure water, could not be recognized. The peculiar red hue of these masses, however, permitted the confirmation of the presence of red blood cells which had been

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softened, partially dissolved, and rendered individually unrecognizable by the water.

Numerous irregular granulations of various hues, some of which were soluble in acetic acid and presented other characteristics specific to microscopic granulations of dust in general were seen between the filaments of hemp or even adhering to their surface.

It is now important to point out that, here and there in the preparation or against the filaments of hemp, were found some cuboidal epithelial cells, some seen face on, polyhedric and regular; others, seen from the side, presented one of their edges. Many were wrinkled and shriveled as often happens; but it was possible to flatten a rather large number of them by pressure of the glass slides. Many of these cuboidal epithelial cells were grouped in epithelial strips or plaques formed by cells overlapping each other. Certain of these plaques or lamellae were pleated or folded over, which rendered the determination of their nature by examination of the constitutive cells a bit more difficult. They can, however, be flattened by suitable movements of the slides, and naturally, flattened cells, easy to study, are also found. Despite their former dessication, these cells can be determined as being finely and uniformly granulated as are those of the vaginal wall and uterine cervix. These greyish rounded granulations, grouped together, are absent in cells of cutaneous epidermis, as is known, or are much less numerous and less regular.

Other than these cells, a few ovoid nuclei of nuclear epithelium are found, finely granulated, about 9 thousandths of a millimeter long, 6 to 7 thousandths wide, similar to those of the uterine mucus.

Addition of acetic acid rendered still easier the identification of these characteristics of nuclei and epithelial cells. This reagent causes the disappearance of blood cells or the masses they form, as well as a part of the granules of dust which mask the cells somewhat. At the same time, it swells and pales the cell and brings the nuclei as well as the fine granulations surrounding them into relief.

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It also renders the epithelial plaques, formed by overlapping cells, easier to study, making the overlapping more easily recognizable, as well as the nuclei of the cells which cover these overlapping cells. It renders strikingly evident the analogy of these epithelial strips with those obtained by lightly scraping the mucosae, as that of the vagina, for example.

After the action of acetic acid, in addition to the finely granulated, nucleated cells of which we have just spoken, a few cells without nuclei were noted, much less numerous, entirely, or almost entirely, lacking in granulations. They preserved on their surface the folds or projecting lines corresponding to lines of juxtaposition of overlapping cells to which they were adherent, peculiarities found particularly in desquamated cutaneous epidermal cells. These last cells were, at the same time, smaller than those provided with nuclei and finely granulated as are those of the vagina and other mucosae.

If it is imagined that the stains observed here were formed on a shirt in contact with the trunk and thighs for a long time, it will be found quite natural to see desquamated cutaneous epidermal cells retained by mucusy bloody stains of the fabric, and mixed with cells of these stains formed of blood cells and vaginal epithelium, or at least similar to that of vaginal mucus. All these characteristics are easy to determine for anyone used to the comparison of epithelia.

The stains whose constitution we have just studied were the least colored of all. They were the color almost of rust or of a reddish brown, staining the linen in the manner of a liquid penetrating by imbibition.

We then studied in the same way stains mixed with the preceding, or neighboring them, presenting an irregular contour, thicker, and truly red.

Now, we found absolutely the same elements as in the less colored stains. The blood cells were much more numerous, but the fibrillary web specific to fibrin could not be discovered in either one or the other. Cuboidal epithelial cells were found in rather considerable quantity, and were especially easy to recognize after addition of acetic acid, as well as ovoid nucleated epithelia, but these were less abundant.

On none of these stains, before or after addition of acetic acid, could be found *mucus corpuscles* or *white blood cells* presenting characteristics distinct enough for a description or a determination of their presence with certainty. These blood stains scarcely soaked through the thickness of the fabric, i.e. scarcely colored the outside surface of the shirt. An anatomic element was nonetheless sought, the presence of which might be useful to determine. A few epithelial cells without nuclei were found here and there with very fine lines projecting from their surface and presenting the other characteristics noted as specific to cutaneous epidermal cells fallen by natural exfoliation. These cells were present in very little number; only about 2% were found on the other surface, on the same portion of the stain which was in contact with the external genitals.

Some rare nucleated cells, similar to those of epidermis, were also found on the unstained parts of the shirt, in the intervals between the stains. But here none of the cells presented finely granulated nuclei analogous to those found in the vagina or the labia minora, whose characteristics were established above.

From the facts observed above, from the presence of elements of blood mixed with those of mucus of the genital tract, i.e., with elements of the mucosal epithelium, we can conclude that these blood stains just studied were actually formed by blood of a menstrual period; for similar characteristics are found in normal menstrual blood. The latter, as is known, is an intimate mixture of blood proper, with mucus furnished by the genital tract and by the vagina in particular. Now, this mucus, holding epithelial cells principally and leukocytes in a more or less large quantity in suspension, causes these elements to be added to those of blood which does not normally contain them in vessels. It is easy to identify them, either in fresh blood or in dried stains, and the nature of these stains thus reveals their origin as well as the organs from which the blood has escaped.

§ V. Examination of small, round, thick blood stains found on a bed sheet. After treatment and examination of these stains in the same manner as the preceding, the presence of red blood cells, white blood cells and fibrin specific to blood stains proper could be determined. These characteristics are so identical to those we have described in analogous stains in our previous memoir on this subject that it is useless to /432 reproduce their description here. (Cf. Robin and Salmon,

loc. cit. §§ IV, V, VI, VII and Briand, Chaude, and Gaultier de Claubry, loc. cit., pages 806, 807 and 808).

But it is important to note that these stains were completely deprived of epithelium, and despite intensive research, no cells whatever could be found, analogous or not to those found so easily in stains actually formed by menstrual blood.

It could be that, in cases where the menstrual period flows abundantly, exfoliated uterine and vaginal epithelium having been carried along, blood accumulated in the vagina during sleep thus falls in thick drops containing only the anatomic elements of blood. Consequently, we cannot conclude anything from the facts observed above in the case at hand, if not that the drops in question are constituted by blood; but it is impossible to determine their origin from the elements constituting them.

It is important to remark that stains thus constituted, found mixed with stains, some of which are deeply colored, others paler, such as those identified in the preceding paragraph as actually formed by menstrual blood, do not invalidate this determination by their presence. A mixture of bloody stains containing only elements of blood and of stains containing, in addition, those of the mucus of the genital tract, does not prove that these stains come from elsewhere than uterine capillaries. Indeed, although observations done up to the present have always showed elements of mucus mixed with menstrual blood, even at the moment of their highest flow, it is understandable that this flow could be quite substantial for a number of hours and produce stains

/433 of pure blood, preceded and followed by the formation of stains containing epithelia of mucus usually found during the total duration of menstruation.

It could also be that blood coming from a wound could form stains on a fabric already stained by menstrual blood. The microscope and the preliminary methods of investigation, which we have previously reviewed, give no less precise results and in each of them find no less the anatomic elements which characterize them, such as we have described. The case excepted where there would be a mixture or superposition of ordinary blood to menstrual blood, these tests can always clarify the investigation in a precise manner.

Menstrual blood, always being somewhat mixed with mucus, it is not astonishing that the stains (as menstrual blood itself) can be distinguished from those formed by pure blood. It is by the continuously exfoliated epithelia which it carries with it, that the mucus is recognizable in the microscope. The origin of any mucus can be determined by the characteristics of the variety of epithelium specific to the mucosa from which it originates. It is by the mixture of uterine and vaginal epithelial cells with blood cells that menstrual blood, and the stains it forms, differ from blood of a wound, and it is by the presence of epithelia that the nature can be determined. Only the esophagus can yield to the blood originating from it an epithelium analogous to that of the vagina, for blood flowing into the stomach is rapidly altered and the characteristics of this alteration can be easily recognized.

§ VI. Conclusions. The preceding research permits the following conclusions:

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1) On examination by microscope, menstrual blood differs from blood drawn from vessels by the mixture of blood cells with epithelial cells and leukocytes (termed mucus corpuscles). The former come from the epithelium of the uterovaginal mucosae and the latter from the surface of these mucosae.

2) The stains constituted by menstrual blood present elements not found in those formed by blood coming directly from vessels. These elements are those which the mucus of the genital tract, carried along with the blood, holds in suspension, i.e. principally the above-mentioned epithelial cells and mucus leukocytes.

3) Stains formed by menstrual blood can be distinguished from those produced by blood flowing directly from blood vessels by comparison of the two types of stains in the microscope, which will demonstrate the preceding characteristics.

On the Certain Recognition of Blood and Bloodstains in Forensic Investigations*

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7295 Blood can occasionally suffer a great alteration in its characteristics, when it has come into prolonged contact with certain substances, so that one is unable to recognize it by a chemical method when using the usual reagents. This is of importance in legal examinations as I have become convinced by my own experiments.

When blood is in a dried state, unadulterated with other substances, and is submitted to testing, it causes no difficulties, even if the amount of dried blood is extremely small. One treats the blood continuously and for a long time with cold, distilled water, pouring off carefully from time to time the water from the undissolved fibrous substance, until that substance is relatively free from blood coloration as a result of the treatment with water. One can clearly recognize the residual fibrous material as such when viewing it through the microscope, especially if one compares it to another sample which has been freshly produced from a small amount of dried human blood by treating it with water with this purpose in mind.

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One tests the watery solution of blood pigment with reagents, using for these experiments only the first, relatively concentrated solution, since the following solutions, which served as wash water, contain too little of the loosened blood pigment. The reagents which one uses are well known and are described in the textbooks of forensic medicine. One heats a portion of the liquid to the boiling point, by which a greater or lesser degree of clotting takes place in the liquid, according to the quantity of dissolved blood pigment. If the solution is very diluted, often only an opalescence forms. The color of the clotting is a dirty red. It quickly dissolves in a heated solution of potassium hydroxide; the color of this solution is more or less green. It has the characteristic, however, that, if the liquid is of a certain dilution which is not too strong, it appears green only in direct light; in reflected light it appears red. One can best make these observations in a reagent glass.

If one adds to another portion of the blood solution plenty of chlorinated water so that the solution smells after being shaken, it will lose its coloration, and white flakes will sepa-/297 rate out, flakes which normally float on the surface. If nitric acid is added to a third part of the blood-pigment solution,

a grey white precipitation forms, and tincture of gallnut produces a faintly violet colored precipitation in the fourth portion of the solution.

All of these reactions cannot be set up if one has only a very small amount of dissolved blood pigment at his disposal; if, for example, only an insignificant blood stain has been treated with water. In this case it is advisable to boil the small quantity of concentrated or undiluted solution and to treat the boiled solution with potassium hydroxide. If one has obtained the results presented above, this alkaline liquid can be added to a large amount of concentrated chlorinated water whereby white flakes separate out. Or one can choose to use only a half of the alkaline solution for this test so that he can saturate the other half with nitric acid in order to obtain the precipitate mentioned above.

The handbooks describe thoroughly how the solutions of blood pigment can be easily and positively distinguished from solutions of other dyes of organic origin by a chemical method, and also how blood stains can be easily recognized when they are found on undyed linen or wool material. One can easily remove the blood pigment by treating it with cold water so that the fibrous material remains on the cloth; it can then be carefully scraped off and observed with the microscope.

If, however, blood stains are found on dyed cloth, or especially on cloth that consists of an organic nitrogenous substance such as wool or silk, these stains can be identified only with difficulty, if they are not present in a significant amount so that the dried blood can be carefully scraped from the cloth. This process goes very well, even in cases of small amounts, if one proceeds with caution. Sometime ago, I had the opportunity to prove this to myself when I had to submit bloodstains to testing, stains which were present only as very fine drops, which had been squirted onto a black cloth shirt. I was able to convince myself of their existence only by examining the area where they were supposed to be located with a good magnifying glass; this process was more successful in lamp light than in sunlight. These drops were scraped off with great caution. In doing this, many of the cloth fibers were naturally also scraped off. The scrapings, which were present only in a very insignificant quantity, were put into a small white porcelain bowl, and cold water was then poured over them. After a rather long period of digestion in the cold water, the liquid took on a reddish hue. The solution was poured off from the undissolved cloth fibers. On account of

^{*} Translation of: "Ueber die sichere Erkennung von Blut und von Blutflecken bei gerichtlichen Untersuchungen".

in Vierteljahrschrift für gerichtliche und öffentliche Medizin 4: 295-310 (1853).

the presence of the cloth fibers, one could not in fact examine microscopically the undissolved fibrin. In the reddish solution, however, a clot was produced by heating-a clot which broke down when boiled in potassium hydroxide into a greenish solution, which clearly displayed the dichroism mentioned above, and which also produced by means of reagents the reactions described above.

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Of very special significance, however, is the examination of blood, when it is dried on metallic iron. In such an examination, I encountered special difficulties which I find are not mentioned in early experiments of a similar type. It seems to me that describing these difficulties is of some importance. importance.

Vauquelin¹ first made the observation that when iron rust formed on objects of metallic iron inside inhabited houses, it contained ammonia. Chevallier,² who confirmed this observation, also found ammonia in the iron oxide which is found in nature. Moreover, Boussingault³ discovered this alkali even in an iron hydroxide which had not come into contact with the air when it was located. Austin had earlier discovered that ammonia formed when iron oxidized by coming into contact with air and water.

Therefore, if one suspects that traces of dried blood could be on a metal cutting instrument whose surface is coated with rust, one must not think he has found a confirmation of this suspicion when ammonia is found upon heating the scrapings of iron rust.

If, after gentle heating in a dry test tube, the ammonia separates from the iron rust, which one has scraped from the iron instrument, then, even if only small amounts of blood /300 are present, strong heating must produce the well known, strong smelling odor of burning charcoal which arises when albumin substances are carbonized. A brown, malodorous, empyreumatic oil will be revealed on the unheated part of the test tube. The supposition can be confirmed with greater certainty, if one mixes a small quantity of gently heated iron rust with approximately the same amount of potassium, or better of sodium, in a very small glass tube, which has been closed at one end beforehand. After cooling, the mixture is treated with water; the filtered solution is adulterated with a very small amount of iron solution which contains both oxide and protoxide. Then it is soaked with some hydrochloric acid. A greater or lesser quantity of ferric ferrocyanide is left undissolved, if blood was present there. The color of the ferric ferrocyanide is green only if the amount of iron solution added was too much.

These phenomena will surely appear when blood is present in the iron rust, even when the blood is present in the very smallest quantities. These phenomena, however, do not necessarily arise from the presence of blood since they are caused by any nitrogenous, organic substance. If, on the other hand, the iron rust formed only because of oxidation of iron by damp air, these phenomena will certainly not occur.

Moreover, bloodstains, which have dried on smooth, metallic iron, can be easily distinguished from rust spots. /301 The former are dark brown and are only a bright red if the

blood has spread itself very thinly in the drying process. Blood spots distinguish themselves especially by the fact that they are easily loosened from the iron after the blood has dried, and they leave the metal relatively clean. Rust spots, on the other hand, are solidly anchored to the iron and can be removed only with difficulty. Thus, when a knife, spotted with blood stains, is stored for a long time as a *corpus delicti*, it is easily possible that, after a certain time, no blood traces can be found on it because the dried blood can easily be removed from the knife by the slightest rubbing and thus be lost.

I have been able to convince myself of these facts through my own experiments in a forensic examination. A knife, which had very probably been used to commit murder, was handed over to me for examination. This murder took place during the summer in a corn field. After the murder, the knife was left lying in the field and was discovered only some time later.

The blade of the knife was heavily coated with rust since it had lain on the moist earth. As a result it was possible to observe the metal surface of the knife only in a few places.

The rust spots looked exactly like rust as it forms on metallic iron under the influence of dampness and air. After the rust had been scraped off and heated in the test tube, it produced ammonia which caused a moistened, red litmus paper to turn a strong blue; but when strongly heated no burnt odor was produced, and no traces of empyreumatic oil were noticable. If the heated rust was fused with sodium, no ferric ferrocyanide could be obtained by the method mentioned above.

The knife in question was such that its blade could have been covered, but it was sent to me with an uncovered blade and was most likely found in this state, either embedded in the ground or lying on it, so that blood adhering to its surface must have been washed away by the rain; the iron rust, forming on the blade, could not contain any of that blood.

The inside of the knife's surface was filled with a dark, almost black substance which, immediately after having been scraped from the knife, was still somewhat soft but subsequently hardened to a fragile mass. When a very small portion of the material was heated in a small test tube, it behaved just like dried blood; a strong burnt charcoal odor developed, a strong smelling empyreumatic oil formed, and from the heated residue, a significant quantity of ferric ferrocyanide could be produced by treatment with sodium.

When water was used to treat a larger quantity of the black material, it did not extract from this material any blood pigment even after prolonged contact. The digestion was continued for a very long time and was even supported by gentle heating; the temperature, however, could not be so high that the albumin-like substances, which were somewhat dissolved, could be coagulated by the process. Despite this operation the water remained completely uncolored. After 3 filtration the use of reagents showed that only a very small trace of albumin-like substances had been absorbed.

If, on the other hand, the black substance was treated with

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water and then cooked with some potassium hydroxide solution, this immediately turned a greenish color; the filtered solution displayed the dichroism mentioned above and reacted with the reagents just as a solution of blood pigment in potassium solution. When the substance was mixed with hydrochloric acid, after treatment with water and with potassium solution, this dissolved a significant amount of iron oxide, which, when the solution was saturated with ammonia, gave a voluminous precipitate.

The black mass from the sheath of the knife thus consisted mainly of dried blood and iron oxide, which had formed as rust on account of the moisture on the metallic iron with which the inner shell of the knife was covered.

Through the presence of a great amount of ferric hydroxide, the dried blood had lost one of its essential characteristics, its solubility in cold water. In fact, according to the many comparative experiments which I set up in the last stages of my work, the wet ferric hydroxide precipitates completely the blood pigment from its solution and hinders the dissolution of the pigment in water.

A further investigation of the contents of the inner surface of the knife in question showed that this was the case. Besides the black material, a very small piece of wood was found squeezed in approximately where the point of the blade can strike in order to prevent this blade from striking against the iron of the inner sheath. Dried blood had adhered to this piece of wood, especially on the one end of this piece, which probably had not come into contact with the metal rust. By viewing it with the magnifying glass, one could clearly recognize it as dried blood.

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This small piece of wood was anchored to a linen thread at its end where the least blood was found. It was then placed in water in a thin test tube so that it could not float on the top of the water but was submerged for the most part. After some time one could perceive very clearly that red stripes sank from the wood, and indeed from the places where the dried blood was located, toward the bottom of the glass, while a flaky, voluminous material remained fastened to the wood, a material which took on a brighter color the longer the water acted on it. After a time a larger portion of this material separated from the wood and fell to the bottom of the glass. After two days the wood sliver was removed from the glass, and the flaky material which still remained fastened to the wood was submitted to microscopical inspection. It proved to be identical with the fibrin, which had recently been produced from dried human blood by a similarly treating it with water for a comparison with preserved blood. The reddish liquid from which the fibrin had separated was poured off. Even though it was a dilute solution, and contained only a little of the dissolved blood pigment, it displayed unambiguously the presence of the blood pigment when treated with the reagents.

In view of the characteristic of ferric hydroxide to bind the blood pigment, and in view of one of blood's most important characteristics, its solubility in water, it deserves considera-

series of tests concerning this aspect, the results of which I want to communicate here in summary.

If freshly precipitated ferric hydroxide is dissolved in the cold, together with a diluted solution of blood pigment, and the mixture is shaken often, after twenty-four hours the filtered solution contains no blood pigment, but cooking the iron hydroxide with potassium hydroxide releases blood pigment which can easily be discovered in the solution with reagents.

If, instead of wet iron hydroxide, red hot iron oxide is treated with a solution of blood pigment, it removes a great deal less of the hemoglobin. After 24 hours the filtered fluid is still colored, but by cooking with potassium solution, a considerable quantity of blood pigment can be extracted from the iron oxide residue.

The sooner the iron hydroxide is used after its precipitation, the quicker it removes the color from a solution of blood pigment; it is, however, difficult in these cases to filter the fluid because at first the iron oxide passes mechanically through the filter system. But if one has filtered the liquid until clear, he can find no trace of blood pigment in it.

A solution of blood pigment is adulterated with a sufficient quantity of ferrous chloride, and then from this mixture iron oxide is precipitated by using ammonia. After this liquid was 306/ filtered, it was colorless and contained no blood pigment. It is understood that, in these experiments, the blood pigment is present in such small quantities that the precipitation of iron oxide by means of ammonia is in no way hindered.

If, on the other hand, the solution of blood pigment is mixed with a solution of sulfuric acid-ammonium oxideferrous oxide (iron ammonia alum), and subsequently with ammonia, the filtered liquid is then not completely clear of coloration, even when the quantity of iron salt is rather considerable. If ammonium chloride is also added to the solution, and then ammonia, the filtered fluid is then completely free of coloration and contains no blood pigment.

If one lets blood pigment dry in an iron container, completely coated with rust, at the usual air temperature, and then moistens the dried mass with water, and again lets this dry, one obtains, after a moderate length of time spent in repeating this drying operation several times, a dry mass which, when treated with cold water, does not transfer to the water any red coloration or any of the blood pigment. If the residue, after being treated with cold water, is cooked with potassium hydroxide, one obtains, after filtration, a deeply colored solution in which the presence of blood pigment can easily be demonstrated by using reagents. The undissolved iron oxide in the potassium solution shows, after dissolving in hydrochloric acid, that it contains iron protoxide when tested with potassium ferricyanide.

If one uses a container with a smooth surface instead of a rusty iron one, and if one lets blood pigment dry in this container at normal temperature, it takes much longer before the blood pigment looses its solubility in water as a result of the build-up of iron hydroxide. One has to repeat tion in forensic examinations. I have, therefore, set up a the moistening and drying process many more times in order

to render the blood pigment insoluble in water. One finally obtains a brown, almost black mass which can be easily pulverized. The substance no longer gives off any blood pigment in the water, and it reacts exactly as the material which was contained on the inside surface of the knife mentioned above.

Aluminum hydroxide behaves with a solution of blood pigment in a similar fashion as does ferric hydroxide. In a freshly precipitated state, it absorbs the pigment, and the filtered solution is colorless, containing no blood pigment. It appears that a greater quantity of aluminum hydroxide than of ferric hydroxide is required in order to remove the blood pigment from a solution of a certain amount of this pigment.

Ferric hydroxide and aluminum hydroxide were not capable of removing so completely hen egg albumin from a solution of it in water, as they could do in the case of blood pigment.

If a diluted solution of blood pigment was mixed with pulverized clay in the cold and was frequently shaken, it took a long time, even a month or longer, to render the fluid colorless. In this process the blood pigment began to decay and developed the well known odor of decaying cheese. The

/308 filtered liquid then contained ammonium chloride in small quantities but no blood pigment. The clay, however, which had been a white color, changed its color into a somewhat dusky hue in a few places. When heated, it colored a potassium hydroxide solution a greenish color. With reagents it was easily possible to discover the presence of blood pigment in this solution.

If, on the other hand, a very concentrated solution of blood pigment in a very small amount of water, is left for a long time in contact with pulverized clay, the pigment could not be absorbed by the clay. It began to decay, and after several months the red color of the pigment has been preserved. Only when the whole solution was diluted with a lot of water and thoroughly shaken, was it possible after some time to remove the blood pigment from the fluid.

Clay also displays the characteristic that it removes blood pigment from water, although to a far lesser degree than does wet ferric hydroxide. In any case the condition is to be considered in forensic examinations.

These observations are in apparent opposition to those in an experiment conducted by Lassaigne. He used a blood stain caused by about ¼ deciliter of animal blood poured onto a surface of fine sand (pave tendre en grès, clay soil?) in order to see after how long a period it was still possible to identify the characteristics of blood on such a surface. He allowed this piece of earth to be exposed to rain and to light in the open air for one month. After this lapse of time the color was pale and greenish, somewhat inclining toward red. The piece was pulverized and was thoroughly leached in

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cold water for twelve hours; it took on a red-brown color as a result of this treatment, and it showed the presence of blood pigment when acted on by reagents.

The residue, which no longer gave off anything soluble in 2. Ann. de Chim. et de Phys., Vol. 34, p. 109 the water, also had a greenish color; after several experi- 3. Ann. de Chim. et de Phys.. Vol. 34, p. 334

ments, it also showed the presence of blood pigment. It was mixed only with a diluted ammonia liquid, and Lassaigne did not heat it with a potassium hydroxide solution, by which procedure the presence of the blood pigment would have proven itself even more clearly.

Lassaigne concluded from his experiments that, by using the normal reagents, one could still recognize blood stains absorbed by earth even after a month. Obviously, the results would have been different if the blood had not been applied in such a concentrated state but in a greater dilution, and if it had been left spread onto it for a period of time longer than a month. Though he did not use completely satisfactory reagents, Lassaigne himself was thus convinced that the earth still contains blood pigment after complete leaching with cold water.

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The discovery of blood pigment is more difficult if the blood pigment solution has soaked into earth which consists of humus-rich garden soil. For a period of several months I dissolved a diluted solution of blood pigment with earth from a flower pot. After this period the filtered liquid was colorless and, when evaporated on platinum, it left only a very little residue, which, however, contained no blood pigment. The soil, leached with water and then cooked with a potassium hydroxide solution, produced a deeply colored liquid which displayed, after filtration, a dark brown hue but did not display the dichroism which is characteristic of the bloodpigment solution in a potassium solution. In this solution, brown precipitates formed after supersaturation with acids, precipitates which displayed the same peculiarities as did those produced by acids in the filtered liquid; these were also the same as those which the garden soil gave off when treated with potassium solution, even when such earth was not treated with blood pigment. In order to recognize the presence of blood pigment in such a potassium solution, which at the same time contains humus in a dissolved state, it is best to mix the potassium solution with a great quantity of chlorinated water, by which process white flakes form in it, as they do in a solution of pure blood pigment (or of other protein-like bodies), while the humus, dissolved in potassium, does not produce these flakes under the influence of chlorinated water.

If, on the other hand, a concentrated solution of blood pigment is mixed th garden soil at a cold temperature, the pigment will not so easily be absorbed by the soil. Thus, even after several months, water, when mixed with this compound, still produces a red solution which contains blood pigment so that, after completely washing the earth with cold water, it would still have contained blood pigment which would have dissolved in a hot potassium solution along with the humus.

References

- 1. Annales de Chimie et de Physique, Vol. 24, p. 99

Concerning the Crystallization of Organic Components of Blood* L. Teichmann

/375 dessicate slightly as a consequence of spontaneous evaporation, and when evaporation is again induced under a cover slip, crystals formed from the blood will be obtained."

With these words, Funke specified the conditions under which, in his opinion, crystals are obtainable from spleen vein blood (see this journal. N.C., Vol. I, page 185). He later used the above method (ibidem, Vol. II, page 289) and succeeded in obtaining crystals from other blood types. The same preparation method was applied with some modifications by Kunde, who believed that the presence of fibrous material constitutes a definite obstacle and that a certain amount of serum is needed (this journal N.F., Vol. II. p. 274). Apart from the disadvantage that the mechanism of the crystallization could not be clarified, the aforementioned method has the additional shortcoming that it is never certain whether crystals will be obtainable. At first, I used the technique for a prolonged period of time as well. I noticed initially that it is impossible to obtain crystals from fresh blood samples. I then attempted to add various volumes of water to the blood without previous blood evaporation; when

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4 to 5 or more parts of water were added to one part of blood, then after letting the liquid stand for a sufficiently long period, and when slow evaporation became evident by the dark red to violet color of the liquid, crystals could be obtained on each occasion. With this method I verified that the blood of all animals investigated by me in all types of blood containers underwent crystallization. The frog blood was the only exception; on the other hand, I detected crystals in human blood as well as in the blood of dogs, steers, hogs, rabbits, pigeons and fish.

I used a small cork prop to support the cover slip from one side, so as to retain larger quantities of the considerably diluted blood; regular crystals then always formed at the three non-supported sides. With this system, I never obtained crystallization of the whole blood, nor was it possible to prepare crystals without a cover slip; the first-mentioned phenomenon led to the supposition that another substance is present, besides the crystallizing component, which does not participate in the crystallization process. The possible presence of such a substance could also explain why no crystallization occurred with free access to air. The a priori probability existed that the fibrin- and albumin material of the blood plasma does not undergo crystallization and does

"When water is added to one drop of blood, starting to not promote the crystallization of any other substance. Kunde, as mentioned earlier, stated that when crystallization is to be obtained, the blood should not contain any fibrin, nor should its serum content be too low. The crystallization is in fact accelerated when the fibrin and part of the serum are eliminated from the blood. Due to its albumin content, serum, like all viscous substances, such as for example liquefied glue, gum arabic, etc., is quite an unsuitable medium for crystallization. Serum evaporation is too irregular and results in a desiccated crust on the surface, which will later show irregular cracks when water evaporation from the interior of the mass continues. When the liquid contains a large amount of albumin, but not much water and common salt, for example, crystalline efflorescences or small lumps are obtained during evaporation at varying degrees of temperature. But fine, large and regular crystals, like those forming when pure water is added at the same temperatures, will never form. Kunde's contention (page 274) that water 37 can be used as efficiently as serum, should be corrected, in that water is much more suitable than serum.

> Since the blood corpuscles contain the crystallizable substance, the latter had to be isolated first, as free as possible from pigment and serum. The currently used and simplest method consists in the filtration of the blood with a sodium sulphate solution. This method could not be applied in our case, since the admixture of another likewise crystallizable substance in large volumes would have been unsuitable for this purpose. I therefore selected the following method: freshly drawn blood was left standing for 48 hours; the clotted blood was then placed on blotting paper, or was rinsed with water; filtration through a linen cloth and later through paper followed. The filtrate, mixed with water, yielded crystals on each occasion. Finer crystals formed proportionately to the successful elimination of fibrous material and serum from the blood corpuscles. Crystallization now took place without a cover slip as well, but the crystals were less perfect in that case. The admixture of water, as in all crystallizations, is useful merely because it reduces the viscosity of the solvent, and prolongs the duration of the time needed for the separation of the dissolved substances from the liquid. The crystallization of the blood corpuscle content can be achieved without water as well; but the resulting crystals will be incomplete and numerous blood corpuscles desiccate jointly with their entire content. The added water evacuates the blood corpuscles and facilitates crystallization in general. It also offers an opportunity for the formation of larger individual crystals.

When we now survey the conditions under which Funke

^{*} Translation of: "Ueber die Krystallisation der organischen Bestandtheile des Blutes".

in Zeitschrift für Rationelle Medizin 3: 375-388 (1853).

and Kunde obtained crystals, it becomes evident thatdefinitely without intending to do so-they complied with the preconditions which we found to be essential. Initially, Funke prepared crystals from the spleen vein blood of the horse. The blood was forwarded to him from Dresden to Leipzig (Vol. I. p. 177), and at least several hours after the animal expired. While he believed that the blood was fresh, the above-mentioned period was nevertheless sufficient for partial blood coagulation. It is quite irrelevant whether or /378 not the blood originated from the spleen vein; he would have obtained crystals after the same length of time from the blood of other vessels as well. On another occasion (Vol. 1. p. 101), he prepared crystals from the blood of dead fish, after finding thick blood coagulations in their abdominal cavity. In that case he obtained more crystals with less difficulty, which he ascribed to a specific crystallization capacity of fish blood, without taking the most significant factor into consideration, i.e. that the blood was already coagulated when collected. After detecting crystals in human blood, (Vol. II. p. 288 and following pages) he attributed this finding to the fact that the container in which he had stored the blood contained a few drops of water; however, he overlooked an important fact: he had investigated the blood only 30 hours after the blood-letting, i.e. after the separation of the fibrin. He examined cat blood 2 days after its collection, saying himself that it was coagulated. He states regarding the hog blood investigation that the phenomena occurred more readily when the blood was left standing for a day. Kunde was likewise able to obtain crystals after eliminating the fibrin, and a small quantity of the serum from the blood.

When a few drops of the blood corpuscle-water mixture, prepared as indicated, are placed on a glass slide, letting them evaporate without a cover slip, crystals will form, in particular when plenty of water was added and the evaporation is slow. However, enough viscous serum components are still present to form a surface crust, in addition to the crystals. The crystals obtained with the above method are unsuitable for microchemical studies because reagents are unable to act directly on the same.

I used the two methods specified below to avoid this disadvantage and to obtain as perfect and best isolated crystals as possible:

1) I placed supporting props under the cover slip on four sides;

2) I allowed the liquid to evaporate under a watch glass after dampening the edges of it with blood which, when desiccated, forms an adequate seal, and releases the vapor slowly through the individual pores.

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I achieved my objective with the application of both methods: the crystallization was perfect, as shown by the large number and size of the crystals, and the crystals were isolated.

same preconditions as the crystallization of common salts.

tallization is wanted, the temperature can be raised, but it should not be too high, so as to avoid the coagulation of the albumin. When blood, containing the full amount of serum and fibrin is to be crystallized, a considerable volume of water must be added and the temperature should be as low as possible.

After determining the conditions needed for crystallization in the blood, I attempted to form crystals from frog blood. I was unable to obtain a quantity of frog blood sufficient for the elimination of fibrin and serum; but crystals nevertheless formed when I mixed the blood with large volumes of water and undertook evaporation at very low temperatures. These crystals differed from those found in other blood types; their number was relatively much lower, but increased when more water was added, followed by evaporation. Finally, the number of the crystals increased to such an extent that these could not be mistaken for crystals originating from any of the salts contained in the blood.

As for the shape of the crystals: it varied significantly. Since I experimented principally with dog blood, I am unable to state how crystals formed from dog blood compared to those formed from the blood of other animals. However, [crystal] form is certainly quite accidental and depends on secondary effects. Not even the crystals from the blood of the same vessel of the dog show identical shapes when they are prepared with different methods. Crystals obtained under a cover glass form needles, rods and plates; those obtained under a watch glass show, in addition to the above shapes, rhomboid and tetragonal plates; the rhomboids even appear at dissimilar angles. Prismatic crystals, pointed at both ends, constantly form from frog blood; I do not contend, however, that their shape will always be the same. Tetrahedrons were recently detected in the blood of guinea pigs. Lehmann, in his recent study of this type of blood, found that besides tetrahedrons, octahedrons and other forms occur as well (Chem. Pharmac. Centralblatt, 1853, p. 98).

Crystals obtained under the cover slip show various shades of red or violet; the color originates from hematin. When a drop of red blood (fresh) is evaporated, red crystals result. On the other hand, when a drop of blood which has turned violet after prolonged storage is evaporated, violet crystals are obtained as well. On one occasion, the liquid under the cover slip, supported by props on four sides, evaporated suddenly; the residue consisted of entirely achromatic crystals, forming fine parallel threads. When crystals form under a watch glass, they are either achromatic or pale yellow. Only the largest crystals are red; these are mostly composite forms. Crystals obtained from frog blood, regardless of whether large or small, are always achromatic. Their behavior in this respect differs entirely from the properties of crystals prepared from other types of blood; they appear as light-colored solids in the red fluid. I now had to face the As for temperature: blood crystallization requires the questions of whether colored and colorless crystals in various shades occur simultaneously and of whether the intensity of Crystallization improves proportionately to the slowness of the color depends on the size of the stained sections, i.e., rate of the evaporation, and vice versa. When rapid crys- whether the crystals which seem achromatic are perhaps

merely very fine plates of colored crystals, showing hardly any coloration due to their fineness. These questions are difficult to answer. I can only say that, when I compared the various shapes on a slide, the color grade of the crystals did not seem proportional at all locations to their profile diameter. Professor Henle, to whom I showed the preparations, likewise expressed the opinion that achromatic crystals do exist. This would lead to the conclusions that the crystallizable material of the blood corpuscles, as such, is achromatic and includes hematin only accidentally when separating from the blood.

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My findings coincide with those of Funke, Kunde and Lehmann regarding the behavior of the crystallizable substance, and of the crystals when exposed to open air and to certain reagents, as well as regarding their decomposability, weathering capacity, etc. The crystallizing mass (the blood corpuscles) can be stored for several months in fluid or dried conditions, refrigerated or at room temperature, without airtight sealing. The fluid yields the aforementioned crystals when evaporated under the watch glass (as far as I was able to verify) after 4 months; putrefaction and the amount of the developing infusoria (ciliates) do not interfere with the crystallization process in any way. The desiccated mass can be liquefied and crystallized at any time. Accordingly, the desiccated mass does not decompose at all, while the liquid substance does not decompose readily.

Admittedly, crystals prepared according to the method customary earlier seem to disappear gradually, insofar as they are covered by the non-crystallized mass, which dries later. These crystals, however, are merely concealed. When a little water is added and when the glass cover is touched, the crystals seem to float underneath the cover; when a large volume of water is added the crystals dissolve, but reappear later following slow water evaporation. Such errors are avoidable when the crystals are prepared according to the two methods described by me. In that case, more reliable observation becomes possible because, as stated, most of the crystals are exposed. The usual temperature changes do not affect the prepared crystals, i.e., no weathering occurs, as mentioned by Funke (Vol. II, p. 290). In Lehmann's opinion (Chemisch-Pharmac. Centralblatt, 1853, No. 7, p. 99) the crystals do not decompose readily; this is confirmed by the fact that the crystals or the mass can be liquefied and recrystallized, as reported earlier by Funke (Vol. I., p. 191 and Vol. II, p. 290).

When chemical reagents are used, it should be taken into consideration that crystals, even those which seem to be pure, might nevertheless be covered with a more or less thick 382 layer of an albumin-like substance which inhibits solvent access and mars the results.

The crystals are water-soluble. The degree of their solubility is not precisely determinable at this time: depending on the serum volume which was added during crystallization, the added water contains more or less albumin. Therefore more water must always be added than the volume required for the dissolution of the crystals alone.

When strong (approximately 89°) alcohol is added, the crystals shrink, their sharp outline and their plane surfaces vanish; most crystals dissolve slowly in diluted alcohol. According to Funke (Vol. I. p. 189), crystals prepared from watery alcohol are merely "crystallographic malformations". Kunde (Vol. II, p. 275) states that the crystals are never quite regular, and according to Lehmann (*Centralblatt*) the planes of crystals treated with alcohol (the alcohol concentration is not indicated) are no longer quite level and the crystals retain their shape only to a more or less limited extent.

The crystals are not soluble in ether, and retain their shape in it. When water is subsequently added, the crystals remain insoluble; but their consistency changes: they become gelatinous. They dissolve readily in caustic ammonia. Concentrated potassium hydroxide does not dissolve the crystals but they are soluble in acetic, hydrochloric and nitric acids.

According to reports so far, and based on Lehmann's recent investigation results, it can no longer be doubted that the crystalline substances, whose reactions are described here, originate from the blood corpuscles and are organic components of them. It is still not quite certain which of the blood corpuscle components is crystallizable. Our earlier statements on the preparation of achromatic crystals indicate that it is, to say the least, highly questionable whether such crystals can be defined as hematin crystals. It is in fact doubtful whether hematin is involved in blood crystallization at all. Another substance, globulin, if one wants to give it that name, constitutes the residue in blood bubbles after the elimination of hematin. Both substances are obtainable in pure condition by means of water extraction from the membranes. However, the medium used to separate them from one another in the watery solution could modify one and possibly both substances; in particular when coagulated globulin forms, crystallization investigations are no longer feasible. I, therefore, used the freshly desiccated blood corpuscle mass. Concentrated hydrochloric acid dissolves the mass (diluted hydrochloric acid has no effect on it); the dissolution occurs more readily at higher temperatures, while gas (carbonic acid) is released. The resulting liquid is clear, purplish-red and precipitates when water is added. At room temperature the admixture of water to the liquid mass will merely cause clouding. When treated with nitric acid, fine gas bubbles will cover the mass, which dissolves slightly at low temperatures and will dissolve completely when the temperature is raised. In the latter case, the liquid stains yellowish-red; when water is added, a dirty yellow precipitate will form.

When sulphuric acid is used, the mass inflates at low temperatures and most of it will dissolve after a prolonged period of time; however, when the temperature is increased, the mass dissolves quite easily and completely, forming a dark brown, clear fluid, while gas is released. Add d water causes minor clouding.

When these acid solutions are neutralized with potassium

or ammonia, a yellowish-brown precipitate forms which is once more dissolved into a clear red fluid in excess precipitation medium.

In a concentrated potassium solution the mass is loosened and floats on the surface. Light red drops of various size are visible under the microscope. The process, however, occurs at higher temperatures only; no modification whatsoever is determinable at lower temperatures. The mass dissolves in a diluted potassium solution and a clear red fluid is obtained.

Ammonia dissolves most of the mass slowly at higher as well as at lower temperatures.

The residues of these solutions, regardless whether the evaporation took place at room temperature or at higher temperatures, show nothing worthy of note under the microscope.

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In acetic acid the mass dissolves at moderate temperatures into a clear, red fluid; however, it will cloud when the temperature is raised and the color changes to a dirty brown. The same phenomena are evident when the mass is treated with oxalic acid, tartaric acid, citric or lactic acid and probably also when treated with other organic acids.

A drop of the above-mentioned fluid, or a minimal amount of the desiccated blood corpuscle mass, yields crystals when dried at 20 to 50° R under a cover slip with any of the aforementioned acids, in particular with acetic acid. The resulting crystals show the following characteristics:

Their color is always yellow, brick-red, brown or black. They form rhomboid columns, either regular or with slightly blunted angles; twin crystals or stars occur frequently, but these are very fine, resembling the needles, rods or grains of the black pigment.

These crystals are not sensitive to the effect of air; they are insoluble in water, ether, alcohol, acetic acid, hydrochloric acid and nitric acid when these substances are added directly; the crystals dissolve entirely when boiled with nitric acid. They will also dissolve in a diluted potassium solution; when the solution is concentrated, the crystals turn black, inflate and their sharp outlines disappear. Concentrated sulphuric acid dissolves them and they are even more readily soluble in ammonia.

Before dissolving the crystals, they should be subjected to careful, isolated examination, since those coated with albumin are difficult to dissolve or do not dissolve at all. Care should also be taken to avoid mistaking the impression (mold) left by dissolved crystals for decolorized crystals.

A yellow ring always forms around these crystals during their dissolution; in particular after treatment with sulfuric acid, color changes will occur.

The crystals described differ significantly from Virchow's *hematoidin* crystals (*Archiv f. Path. Anat.*, Vol. 1., p. 383-445), but some similarities do exist. The main difference is that the crystals described here dissolve completely in the reagents mentioned, without leaving any residual structure of insoluble substance behind. I propose the name *hemin* (*Hämin*) for the substance obtained in crystalline form by me, to distinguish it from the water-soluble hematin of fresh

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blood and Virchow's hematoidin.

I obtained hemin crystals from all blood types which I examined, including human blood, and the blood of the dog, rabbit, steer, hog, pigeon and frog; no dissimilarities whatsoever were determinable and I do not doubt that the crystals can be prepared from any red blood type.

They can also be obtained on a large scale with the following method: blood—regardless whether or not fibrin and serum were eliminated—is evaporated until dessicated; excess concentrated acetic acid is added and the liquid is left standing at the approximate temperature of 30°. After some time, complete dissolution takes place and the crystals remain at the bottom (of the container).

The preparation according to the specified method seems easy; but it involves a certain difficulty, since microscopic crystals can seldom be separated from the liquid by filtration. The crystals could, of course, be allowed to precipitate and could be suspended in water after distillation of the liquid. But this procedure is deficient, in particular when the quantitative determination of crystals in the blood is intended. I obtained the largest and best-shaped crystals when mixing dried, finely pulverized blood with a large volume of acetic acid under constant agitation, so as to avoid the formation of clots; the liquid was then left standing at a temperature of approximately 30°R. The difficulties increased when the desiccated blood is processed with other organic acids, such as oxalic, tartaric, citric or lactic acid. I undertook only occasional tests with these acids and always obtained very fine rods and grains, but the crystals were never as large and perfect as those obtained after treatment with acetic acid.

Regrettably, I have had no opportunity so far to undertake a thorough investigation of crystals present in old extravasations and melanotic tumors. The presence of crystallized hemin in the organism is, in my opinion, highly probable, for the following reasons: water is eliminated by resorption from coagulating blood; the needed heat prevails, and provided that any organic acid is present as well, the preconditions for the formation of crystals do exist.

I searched unsuccessfully for the crystals in lymph and serum first described by Funke but could detect no trace of them with any method. I found hemin crystals in the lymph and serum, but always in small amounts only. I therefore suspect that they might originate from a few blood corpuscles which were accidentally introduced into both liquids. Provided that color is not essential, the first crystals could consist of globulin (?) or this substance could at least contribute primarily to their formation, since no other substance occurs in the blood corpuscles in sufficient quantity for the formation of numerous crystals. The hemin crystals seem to originate from hemin alone, since the albumin-type substances remain dissolved after treatment of the desiccated blood corpuscles with acetic acid. But it would not be justified to speculate here on the nature of the lastmentioned crystals, since they can be prepared on a large scale and can be subjected to accurate chemical analysis.

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As mentioned above, hemin crystals appear in the shape of fine black rods and grains, very similar to melanin; large quantities of these crystals develop, in particular, when a small amount of acid is added to a large amount of desiccated blood. This leads to the question of whether a correlation between these crystals and melanin exists. The aforementioned reagents, however, affect small and large crystals alike while melanin-referring to its characteristic reactions only-is decomposed during boiling by concentrated nitric acid and dissolves incompletely in diluted alkali after prolonged digestion. According to these differing properties, the fine crystals should definitely not be identified with melanin, despite the fact that they are evidently similar to the latter. Therefore the question arises whether crystals formed under different conditions could behave as does melanin, or whether they adopt the characteristics of melanin after a specific treatment.

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I can answer the first question only insofar as I have found that the application of the above-mentioned organic acids to desiccated blood always resulted in the formation of more or less perfect crystals which showed identical behavior when treated with chemical reagents. I determined the following concerning the second question: these crystals, when prepared in adequate quantities, appear black with a blue tint to the naked eye. But when they are placed on a glass slide heated with a red-hot iron, the blue stain suddenly disappears, the crystals turn black as a consequence of carbonization; when boiled with concentrated nitric acid, the black color turns yellow; the crystals then dissolve into a yellow fluid in which sporadic yellow grains and drops of various sizes still float. Digestion with aqueous potash did not dissolve the crystals.

The degree of solubility of the crystals in these reagents is reversed proportionately to the degree of the carbonization.

The presence of black crystals in the organism was confirmed by numerous findings; the possibility of slow carbonization cannot be excluded.

The ash of the crystals is pink; the ash of melanin is whitish-yellow, occasionally red (see references in Virchow, *Archiv f. Path. Anatom.*, Vol. 1., p. 434). Under the microscope, the first-mentioned ash shows large, but rudimentary crystals, while the last-mentioned ash forms non-measurable dots. Could the comparatively significant difference between volumes be responsible for color differences in this case?

When the black crystals verified by MacKenzie, Guillot,

Virchow and others in melanotic tumors are present in addition to black pigment, it is reasonable to assume that the crystals developed simultaneously with the black pigment. The reduced solidity of the tumors might offer an opportunity for the coagulation of molecules into voluminous masses. When grains of various size, fine rods, irregular and finally, regular crystals are prepared in a retort under the same chemical conditions, all show the same behavior. These crystals undoubtedly formed while inhibited by mechanical effects; their development stopped while they were still incomplete. Nor can a discrimination be made between the black grains and rods found in melanotic tumors, lungs, etc., and sporadically forming crystals, since all these formations behave identically under the effect of chemical reagents.

According to the experiments performed by Virchow and others, the pigment in the mass, as its color darkens, dissolves less easily in potassium hydroxide. Black pigment and black crystals are completely insoluble. When their solubility is compared with the solubility of the hemin crystals obtained by me—regardless whether the crystals form rhomboid columns, rods or grains—it becomes evident that the behavior of both is entirely identical when treated with potassium hydroxide. Non-carbonized crystals dissolve readily, while the carbonized crystals are quite insoluble. Various degrees of solubility can occur between these two extremes.

The last-mentioned difference, found between carbonized crystals and ocular melanin, cannot be defined as significant; since the black color of the crystals is slate-like, while the color of melanin is brown, the difference is explained by the presence of various intermediate pigmentation stages, not found in crystals. The color of the ocular pigment does in fact approximate the color of synthetically prepared pigment.

I therefore feel justified in reaching the conclusion that the black pigment represents underdeveloped crystals, oxidized to various degrees.

I must leave the further study of the subject's chemical aspect to professionals. I wish, however, to refer to the practical usefulness of the knowledge of hemin crystals, namely that they serve in legal cases for the reliable and easy verification of minimal blood volumes, for example in suspected (blood) stains.

Finally, it is my welcome duty to thank Professor Henle for his advice in the course of this study, and for making the facilities of the Anatomy Institute available.

Preparation of Hemochromogen Crystals*

Dr. Zacharlas Donogany

Physiological Institute at the University of Budapest (Received by the Editorial Office 24 December 1892)

Hemochromogen or reduced hematin is prepared by /629 means of different processes which are, however, quite difficult. Hoppe-Seyler[†] prepared hemochromogen by heating to 85°-100° the hemoglobin solution and excluding oxygen with strong alkalis; he also used carbon monoxide hemoglobin for this purpose. Recently, Trasaburochraki[§] pre-7630 pared hemochromogen from sulphur-methemoglobin by

means of sodium hydroxide and ammonium sulfide.

In my process I first mix a drop of defibrinated blood with a drop of pyridine on the microscopic slide; I then cover the mixture with a cover slip and examine it by means of a spectroscope and a microscope. The blood corpuscles disappear and the drop becomes a lively brownish-red. In the spectrum, two very beautiful absorption bands appear; one sharply outlined band between Fraunhofer lines D and E, and a brighter, but less well-defined, band between lines E and b. In a thicker layer these two bands merge together into one; in a diluted solution, only the first of the two lines is perceptible.

When I reduced the blood with ammonium sulfide, or even without it, there very soon appeared small, light or dark brownish-red, star-shaped or sheaf-shaped hemochromogen crystals which formed groups. I was unable to examine these

- in Centralblatt fur Physiologie 6 (21): 629-630 (1893).
- Reprinted with the kind permission of Verlag Franz Deuticke, Vienna.
- [†] Zeitschrift f. physiol. Chem. XIII.
- [§] Zeitschrift f. physiol. Chem. XIV

crystals spectroscopically because of their small size. There can, however, be no doubt that these are hemochromogen crystals, since they always occur in the hemochromogen which forms the basic substance, and also because the crystals disappear if the hemochromogen changes into hematin. The preparation of hemochromogen crystals is also successful with old, dried blood if one pretreats the blood with sodium hydroxide.

These doubly refractive crystals are not stable because, when air penetrates, the red hemochromogen changes into brown hematin, at first around the edges, and then disappears completely after several days; the spectrum then corresponds to that of alkali hematin. If, however, one seals the edges of the cover slip with Canada balsam, then the hemochromogen crystals can be preserved for a longer period.

One can also demonstrate the changing of hemochromogen into hematin by the following method; One can prepare hemochromogen by means of pyridine from defibrinated blood, diluted with water in a test tube. If one transfers a half of the solution into a second test tube and shakes this up with the air, one can see how the red hemochromogen solution loses its red color in a few minutes, and how the hemochromogen changes into brown hematin. The spectroscopic diagnosis also changes in a corresponding fashion.

My method, consequently, permits one to prepare hemochromogen in an easier and faster way than was formerly possible; it is at the same time suited to establish more easily, and perhaps with greater certainty, the presence of blood in dry powder than is possible by means of hemin crystals.

^{*} Translation of: "Darstellung von Hämochromogenkrystallen".

A Method for Identifying Blood by Hemochromogen Crystallization* Masao Takayama, M.D.

/15 demonstration of:

A. Blood corpuscles, especially erythrocytes, and

B. Blood pigment and its derivatives.

Blood pigment and its derivatives are in turn identified by the following three methods:

1. testing catalase and oxygen-combining activities,

2. examining crystals, and

3. studying spectra.

Among these three methods, the first one that tests catalase and oxygen-combining activities is applied mainly in clinical cases for identifying blood because of its simple

/16 procedures and extreme sensitivity. Every year its improvements or modifications are published by clinicians. Unfortunately, however, the reactions demonstrated by this method are not specific to blood pigment and its derivatives; as you are well aware, various organic and inorganic substances give the same reactions. Therefore, we regard this test method as preliminary or auxiliary to the identification of blood: a positive result does not necessarily indicate the presence of blood pigment, but a negative result does prove its absence.

The second and the third methods, the examination of crystals and spectra, are indispensable to forensic medicine for the identificaiton of blood. The positive results absolutely prove blood pigment and its derivatives.

The blood pigment and its derivatives that form crystals are: hemoglobin, oxyhemoglobin, methemoglobin, carboxyhemoglobin, cyanohemoglobin, sulfhemoglobin, hemochromogen, carboxyhemochromogen, cyanohemochromogen, hematin halide, hematin combined with organic and inorganic acids, and hematoporphyrin hydrochloride. Two of these crystals, hematin halide, namely hemin, and hemochromogen, are used for identifying blood, and today I am going to talk about the hemochromogen crystals.

Ever since Teichmann discovered hemin crystals (hence also called Teichmann's crystals) in 1853, the hemin test method has occupied an important place among methods for identifying blood. Hemochromogen was crystallized for the first time in 1889 by Hoppe-Seyler. He crystallized it by dissolving hemoglobin in an aqueous solution of sodium hydroxide and heating it to 100°C in the total absence of oxygen. In 1893 in a physiology laboratory in Budapest,

* Translation of: Original Article-[Japanese Title Not Transliterated].

Clinically and medico-legally blood is identified by the Donogany accidentally synthesized hemochromogen crystals by adding pyridine alone, or pyridine together with ammonium sulfide, to defibrinated or dried blood. These findings remained largely unnoticed by scholars, although R. Kobert's nephew, H. U. Kobert, and Angelo de Dominicis duplicated Donogany's experiments in 1901 and 1902, and later Cevidalli, de Dominicis, and Lecha-Marzo published works on this subject. However, the method finally drew 17/ general attention after Bürker reported in 1909 that it was possible to identify hemoglobin and its derivatives by hemochromogen crystallization. Thanks to the experiments by such workers as Puppe and Kurbitz, Kalmus, Mita, Lochte, Methling, Dilling, Hummel, and Heine, this method has been recognized as the most important among the methods of blood identification.

> The reasons that the methods of blood identification by hemochromogen crystallization has come to occupy such an important position are:

> 1. The crystallizing ability of hemochromogen is equal or superior to that of hemin.

> 2. Because hemochromogen crystals are large and their color varies from copper red to deep ruby red, and detecting them is easier than detecting small, brown hemin crystals. For example, combined with a No. 2 or 4 ocular ($3 \times$ or $6\times$), a No. 7 objective ($60\times$) is needed for detecting hemin crystals, while No. 3 (10 \times) is adequate for hemochromogen crystals.

> 3. Regardless of the success or failure of hemochromogen crystallization, the hemochromogen produced can easily be identified by the spectrum using a microspectroscope or a hand spectroscope (remove the ocular of a microscope and insert a hand spectroscope to examine). According to de Dominicis the hemochromogen spectrum shows the first and the second absorption bands at the dilution of 1:5,000, while 1 cm thickness of oxyhemoglobin solution gives the first and the second absorption bands at 1:5,000 dilution, and the first band only at 1:10,000 dilution. Thus the light absorbing sensitivity of hemochromogen is almost identical to that of oxyhemoglobin.

The disadvantage of this method is that the slide preparation of hemochromogen crystals cannot be preserved long. If the edge of the cover glass is sealed with Canada balsam or masking lac, however, the slides keep comparatively well. Besides, since the purpose of forming the crystals is to identify blood, I do not think it matters much if the slides keep well or not.

1. How are hemochromogen crystals made? Generally speaking the reagents used in this method are:

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in Kokka Igakkai Zasshi, No. 306, Pages 463-481 (cumulative) Pages 15-33 (issue) (1912).

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Identification of Blood

(a) as dissolving agent of dry blood—distilled water, concentrated aqueous solutions of sodium and potassium hydroxide, 10% ammonia water, concentrated potassium chloride solution, 2% alcoholic or aqueous solution of iodine, bromine water, or chlorine water,

(b) pyridine, piperidine, or their derivatives (α - and β -picoline, collidine, parvuline, coniine, nicotine, methylpiperidine, ethylpiperidine, etc.)

(c) as reducing agent—ammonium sulfide (Donogany), saturated aqueous solution of hydrazine sulfate (de Dominicis), saturated solution of sodium antimony sulfate (de Dominicis), 10% hydrazine hydrate solution (Mita), or sodium sulfate.

Among these reagents pyridine is indispensable. My guess is that the dissolving and the reducing agents transform blood pigment into hematin and then reduce the latter to hemochromogen, which crystallizes in the presence of pyridine. Since pyridine obviously does not have a reducing action, it has been disputed whether pyridine is the one that produces hemochromogen: von Zeineck, Kalmus, and Kurbitz maintain that hemochromogen cannot be produced with pyridine alone, while Donogany, Kobert and Dilling claim that it is possible to make hemochromogen and its crystals from fresh blood using only pyridine if oxygen is completely excluded. The slides exhibited will convince you that hemochromogen crystals are certainly produced using pyridine alone. In any case, it is an indisputable fact that pyridine plays a major role among the reagents used in this method.

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2. While the procedures of this method vary somewhat according to researchers, the representative four are:

(a) Donogany's procedure—blood + a drop of pyridine + a drop of ammonium sulfide (or pyridine alone; or concentrated sodium hydroxide solution + pyridine; or concentrated sodium hydroxide solution + pyridine + ammonium sulfide).

(b) Angelo de Dominicis's procedure—blood + a drop of pyridine + a drop of saturated hydrazine sulfate solution or a drop of solution containing 5% sodium hydroxide and hydrazine sulfate (Puppe and Kurbitz mixed blood with pyridine and a saturated aqueous solution of hydrazine sulfate, then heated the mixture to gradually evaporate, and made a permanent slide by sealing it with Canada balsam; Heine recommended a mixed solution containing 2 parts of pyridine and 3 parts of concentrated aqueous solution of hydrazine sulfate.)

(c) Lecha-Marzo's procedure—blood + 2% alcoholic or aqueous solution of iodine, chlorine water, or bromine water, heat the mixture and then add pyridine + ammonium sulfide (although Lecha-Marzo called the crystals thus produced hematin iodide, hematin chloride, and hematin bromide, Kürbitz's research made it clear that they were hemochromogen crystals).

(d) Mita's procedure—blood + a drop of 10% ammonia water + a drop of pyridine + a drop of 10% hydrazine hydrate solution or saturated aqueous solution of hydrazine sulfate.

Though with all these procedures hemochromgen crystals are produced without heating, the researchers are agreed that careful gentle heating increases the efficiency of crystallization.

Such is the summary of the present state of the method for identifying blood, namely blood pigment, by means of hemochromogen crystallization. Today at this meeting I should like to present a new reagent that I have added to these test procedures based on hemochromogen crystallization. My reagent differs from those of others in that I used glucose as the reducing agent. Why did I use glucose? It happened fortuitously. As you know there is a method called Heller's for testing blood in urine. In this method urine is made 24 strongly alkaline by adding sodium hydroxide solution and then it is boiled to precipitate blood together with phosphates. The blood pigment in this brownish red precipitate is called hemoglobin or hematin by different workers, or even hemochromogen by Arnold. One day in order to determine which was the case, I added blood to a urine sample which happened to be on my desk and proceeded with this method. Unexpectedly the blood pigment turned deep ruby red and remained dissolved without precipitating. The pigment had the spectrum of hemochromogen. Puzzled, I tested the urine and found that it was diabetic. From this experience I learned that, in the presence of sodium hydroxide, glucose reduces blood pigment and changes it into hemochromogen. Therefore, I deduced that if blood is first mixed with a solution containing glucose and sodium hydroxide and then with pyridine, and the mixture is heated, the blood pigment would even more easily be changed into hemochromogen, and on a slide its crystals would be formed. I carried out this experiment, which turned out to be successful as I expected.

I repeated the experiment in order to find out what proportion of glucose to sodium hydroxide solution to pyridine would make a favorable reagent for crystallizing hemochromogen. The favorable reagent was found to contain 0.5% glucose, 1% sodium hydroxide, and 10-20% pyridine. Namely:

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glucose	0.5
sodium hydroxide	1.0
distilled water	90.0-80.0
pyridine	10.0-20.0
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10% glucose solution	5.0
10% sodium hydroxide solution	10.0
distilled water	75.0-65.0
pyridine	10.0-20.0

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When the amount of pyridine in the reagent is small, 2 hemochromogen forms small but regular, long, diamondshaped crystals, which are similar to hemin crystals; when the amount is large, the crystals are very large but irregular, most of them being needle-shaped. The needles cluster in the form of a cross, a tassel, or a chrysanthemum flower. Therefore, taking the value halfway between 10 and 20%, I regard the one containing 15% pyridine as the most favorable. (Let us call this the first glucose reagent.)

The procedure for crystallizing hemochromogen with this reagent is extremely simple: a test object is placed on a slide glass, broken into fine pieces with a glass rod, mixed with a drop of the reagent, covered with a cover glass, and heated carefully until gas bubbles appear in the liquid. For heating, an alcohol lamp or the small flame of a gas lamp that is obtained after turning off the valve may be used at a considerable distance. If the object is, or contains, blood, the blood pigment which is brown at first gradually becomes red and. under the microscope, begins to crystallize two or three minutes after the slide is cooled. After 10 to 20 minutes almost all the blood pigment in the preparation has turned into crystals. I just said that the object is broken to fine pieces, but this is a matter of degree: either too large or too small pieces are undesirable. According to my experience hemochromogen crystals start to grow from the periphery of comparatively large granules and reach the centers to form aggregates; if the granules are too small, blood pigment is leached and sometimes fails to crystallize though it may remain in solution. Thus the favorable size of fragments must be determined by trial and error. I should say a mistake of leaving them too large is preferable to that of making them too small. The same can be said in using ammonium sulfide or hydrazine reagents. When a piece of cloth or paper stained with blood must be tested as it is for reasons such as the quantity of blood being too small or the separation of blood being impossible, if the material is thin like thin silk, Japanese Mino paper, and newspaper, a small piece should be cut out, placed on a slide glass, treated with a drop of the reagent, covered with a cover glass carefully so as not to

/22 introduce air bubbles, and heated. If a thick material such as cotton cloth, Chinese silk crepe, or flannel is to be tested, the tissue should be teased with needle tips following the addition of the reagent, covered with a cover glass, heated, and examined under the microscope after the slide has cooled. The iris diaphragm opening of the microscope should be fairly large. Even when the object has a blue, yellow, or red color, hemochromogen crystals are easily detected; only in cases of indigo-dyed fabrics the test fails if the quantity of adhered blood is small.

According to test-tube experiments in which blood was converted into hemochromogen with glucose, sodium hydroxide solution, and pyridine, the heating needed for this method is 80°C. There is no need to heat the slides very much; when over-heated, the preparation may boil, and the dissolved blood pigment could be lost by boiling over. Although great care is needed not to over-heat, reduction will be incomplete and crystals not formed if under-heated. As in using any other methods, some preliminary practice is necessary in this case to learn how to heat the slides.

Since considerable care is thus necessary in heating, I thought that an increased amount of glucose in the reagent might make heating unnecessary for crystallization because of an increased reducing action. I prepared 30% glucose solution, added sodium hydroxide solution and pyridine to it,

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and made some experiments with this reagent. As I had conjectured, hemochromogen crystals were produced just by adding this reagent, without heating. Then in what proportion should the ingredients be mixed to give the best result? I tried various proportions and confirmed that the following mixture always gives good results. (Let us call this the second glucose reagent.)

30% aqueous solution of glucose (glucose 3.0,	
distilled water 7.0)	10.0
10% sodium hydroxide solution	3.0
Pyridine	3.0

Strangely, this reagent works better when aged one or two days to take up an orange-yellow or light brown color than when freshly made. With a fresh reagent, the crystallization of hemochromogen takes from 20 or 30 minutes to several hours after the addition of the reagent, depending on the room temperature. With a one day old, orange-yellow reagent, however, blood becomes red and crystallization begins as soon as the reagent is added and a cover glass placed. Within 10 minutes to one hour almost all blood pigment changes into crystals. The effective period of the reagent with high glucose content is short, however, being about two weeks after preparation. As its color becomes dark brown, the action becomes weaker; after one month the crystallization of hemochromogen takes two or three hours, and fewer crystals are formed. If a slide prepared with a fresh reagent is heated, crystallization is of course aided, but numerous colorless fine granules are also produced to obscure the field of view. Thus although this reagent produces hemochromogen crystals well without heat treatment, it is inconvenient in that it must be prepared at least one day in advance because otherwise crystallization takes a long time to begin. To remedy this inconvenience, I heated slightly a freshly prepared reagent in a test tube until it turned very light yellow, let it cool, and left it until it became light orange-yellow. Such a reagent reacts with blood stains as quickly as the aged reagent does: blood stains become immediately red and numerous crystals appear. The only difference is that the crystals are smaller than those produced with either a fresh reagent or a one- or two-day old reagent. For artificial aging it is better to heat the reagent moderately because over-heating gives it a strong color and reduces its effectiveness.

As I mentioned earlier, I hypothesized that my glucose reagents produced hemochromogen crystals from blood pigment by the following process: by the action of sodium hydroxide solution, blood pigment becomes alkaline hematin, which is reduced by glucose and at the same time is crystallized by pyridine. To test this hypothesis I prepared the 24/ following reagents:

	No. 1	No. 2	No.3	No. 4	No.5
10% glucose solution	0.5	0.5	-	-	-
10% NaOH solution	1.0	1.0	1.0	1.0	-
distilled water	7.5	8.5	7.0	9.0	8.0
pyridine	2.0	-	2.0	-	2.0

Identification of Blood

Of these reagents No. 2 and No. 5 changed blood pigment into hemochromogen but failed to crystallize it; in contrast No. 3, which contained sodium hydroxide solution and pyridine, produced hemochromogen crystals without heating almost as well as No. 1, which was made of glucose, sodium hydroxide solution, and pyridine. Thus, contrary to my expectation, without added glucose, sodium hydroxide solution and pyridine used together produce hemochromogen crystals perfectly well, as Donogany had claimed. Is glucose then unnecessary in my reagents? To answer this question I prepared the reagents listed below and proceeded with the experiment on the following day.

blood-stained materials. Therefore, in spite of the trouble of heating and the danger of losing dissolved blood pigment from the covered area when carelessly heated—this can be avoided by exercise of care, I recommend the first glucose reagent which contains a small amount of glucose. In contrast to the second glucose reagent that is effective for only about two weeks, the first glucose reagent can produce the crystals for more than a year, as long as the bottle is tightly stoppered. From this point, too, the first glucose reagent is superior to the second one. The periphery of the slide preparations made with an old first glucose reagent looks green, suggesting that the glucose has changed and lost its reducing

	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8
30% glucose sol.	10	10	-	_	-	-	-	-
10% NaOH sol.	3	3	3	3	3	3	3	-
pyridine	3	-	3	3	3	3	-	3
distilled water	-	3	10	-	-	-	13	13
10% hydrazine								
hydrate sol.	-	-	-	10	-	-	-	-
saturated hydrazine								
sulfate sol.	-	-	-	-	10	-	-	_
simple syrup	-	-	-	-	-	10	-	-

The results of the experiment were as follows: two or three hours after No. 3 and No. 6 reagents were added to dry blood, the periphery of the preparation looked brownish green while in the inner part the centers of blood aggregates became red and gradually produced hemochromogen crystals. On the other hand, the reactions with No. 1, 4, and 5 reagents, which contained glucose, hydrazine hydrate, and hydrazine sulfate, were almost instantaneous—as soon as the reagents were added, dry blood turned deep ruby red and crystals were formed. From this it is clear that the efficiency of crystallization is increased with the addition of even a small amount of reducing agent, glucose in my case. Thus although heating with sodium hydroxide solution and pyri-

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dine produces hemochromogen crystals, the presence of glucose no doubt facilitates the reaction, as I had expected. The next question is: which is to be recommended as the

reagent for hemochromogen crystallization, the first one with a small amount of glucose or the second one with a large amount? Both reagents produce hemochromogen crystals well, and their crystallizing action is strong. The difference is that one needs heating while the other does not. In cases in which it is possible to separate dry blood from cloth or paper for testing, the second glucose reagent with a large amount of glucose works better. However, if it is impossible to separate blood, and bloodstained cloth or paper must be tested as it is for the identification of blood pigment, the first glucose reagent with a small amount of glucose is more favorable. When used as I explained in the part on the procedure, it produces hemochromogen crystals very well. With the second glucose reagent, although hemochromogen crystals are produced if the test objects of such kind are about one year old, the results are generally not very good. As I have already mentioned, the first glucose reagent produces the crystals well also from dried blood separated from

power because of its small amount. Therefore, in practice a fairly fresh reagent should be used.

Pyridine in my glucose reagents may be substituted with piperidine (20-30%); the reagents still produce the crystals as Cevidalli and Dilling claimed. According to my observations, however, the crystals thus produced are small and somewhat difficult to detect. So I believe that pyridine suits our purpose better than piperidine. I also tried substituting glucose with other sugars. Lactose, fructose, galactose, and honey all function well in the place of glucose. Potassium 26 hydroxide can substitute for sodium hydroxide, but the latter seems somewhat better. However, if sodium hydroxide solution is replaced by ammonia water, my reagents lose their action. According to my experience, even among hydrazine hydrate and hydrazine sulfate reagents, those containing sodium hydroxide solution give better results than those without it.

Last year (1911) Leers reported that when 50% solution of hydrazine hydrate was added to hemin crystal preparations, hemin crystals were reduced to become deep ruby red. He called the product reduced hemin crystals. Heine wrote in the recently arrived Viertelj. f. ger. M. (vol. 43, p. 268) that the mixture of two parts pyridine and three parts concentrated aqueous solution of hydrazine sulfate had the same action as 50% hydrazine hydrate did. I treated hemin crystal preparations with the following reagents according to Leers' and Heine's procedures: my glucose reagents, 10% ammonia + 10% hydrazine hydrate, and a similar mixed solution containing saturated aqueous solution of hydrazine sulfate in the place of hydrazine hydrate (Mita), 1 part of ammonium sulfide + 1 part of pyridine (Donogany), 10 parts of (sodium hydroxide 5.0 + hydrazine sulfate 5.0 +water 100.0) + 5 parts of pyridine + 10 parts of water (modified de Dominicis), and pyridine + sodium hydroxide

solution (control reagent). The results showed that the reagents containing hydrazine hydrate or hydrazine sulfate, or both ammonium sulfide and pyridine, produced what Leers called reduced Teichmann's crystals. However, the hydrazine reagents had the regrettable characteristic of producing in the slide numerous minute gas bubbles that obscured the field of view. Leers' crystals did not keep very long: under continuous observation the pigment was leached from the red crystals leaving colorless residue. My second glucose reagent also reduced the crystals and blood pigment hematin in the preparations, but the red crystals that resulted decomposed immediately, and simultaneously numerous hemochromogen crystals appeared. In this experiment two slide preparations particularly drew my attention: the one that was treated with my first glucose reagent containing a small amount of glucose, and the other that was treated with the control reagent containing one part of 10% sodium hydroxide solution, two parts of pyridine, and seven parts of distilled water. Left at room temperature without heat treatment, the former showed gradual reduction and was red all over after one or two hours. It revealed numerous hemochromogen crystals under the microscope. In contrast the latter looked brownish green and showed no hemochromogen crystals; in the center of the lumps of blood pigment that had failed to become hemin crystals, however, a red color and a few hemochromogen crystals sometimes developed. The contrast between these two slides was quite pronounced, and it proves what I stated earlier, namely, that glucose in my reagents plays a considerable role in the formation of hemochromogen crystals. Also noteworthy is the fact that, in cases in which blood pigment has changed into

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though it takes somewhat long. I should mention here Florence's work in which he used honey to remove indigo that obsured the spectrum of blood pigment. He reported that, if blood stains in an indigo-dyed fabric were treated with a small amount of honey and then soaked in 33% potassium hydroxide solution and blood was reduced by honey to become hemochromogen, which could be detected by the microscope or the spectroscope. Although hemochromogen was produced by this method, its crystallization did not take place. Florence used honey, and I used glucose, to reduce blood pigment; the purposes were different but the ideas were quite similar. This work by Florence was found in Leers' lecture called the present trend in medico-legal blood testing given at the meeting of Prussian Medical Doctors Association on April 27, last year. Since it gave no reference we cannot read the original. As I mentioned earlier, my reason for using glucose was quite unrelated to Florence's work. Although I report on these glucose reagents for the first time today, I already applied this method to identify blood pigment in January of last year when I was ordered by the Kurume District Court to test blood stains related to a murder case. I described the method in the written expert opinion that I submitted on March 6.

hematin, my first glucose reagent produces hemochromogen

crystals without heating in spite of its small glucose content,

Thus I learned about Florence's use of honey for hemochromogen production long after I had used glucose for hemochromogen crystallization. This is beside the point but 28/ I mention it.

In the method of identifying blood pigment by producing hemochromogen crystals, how do my glucose reagents compare with other published reagents? Other reagents have some advantages and disadvantages, as I found out in the comparative study that I made using the reagents published by Donogany, de Dominicis, and Mita. In one part of the study I used the chemicals exactly as the authors described; in the other part I mixed the chemicals to make solutions, and when they failed to mix sufficiently I added some distilled water. The reagents used are given in the table below. Except for ammonium sulfide and 10% ammonia water, all the chemicals I used for this experiment were made by Merck, Darmstadt. I should also mention that, in this comparative study, I heated the slides with great care after adding reagents because careful heating always gives better results.

1. Mita described in detail the disadvantages of the rea- 29/ gent that consists of one drop of pyridine and one drop of ammonium sulfide, which were used by Donogany and others: (1) the yellow color of ammonium sulfide makes the detection of hemochromogen crystals difficult by obscuring their cherry red color; (2) sulfur crystallizes out around the cover glass; (3) an unpleasant odor is given off; (4) if blood stains are on metals, especially iron, the detection of the test crystals becomes difficult or impossible because of numerous, strongly colored crystals of sulfide that are produced. Hummel stated that sulfur crystallized during the heating process to obscure the field of view and that the crystallization of sulfur might obstruct that of hemochromogen. Methling also wrote that sulfur from ammonium sulfide formed crystals and made the slide yellow and difficult to examine.

2. In de Dominics' and Mita's reagents, hydrazine hydrate has a very strong action in reducing blood pigment into hemochromogen. When treated according to Mita's procedure or with the mixed solution, dried blood turns instantaneously red and the blood pigment becomes hemochromogen. But it does not produce hemochromogen crystals as well as hydrazine sulfate does. Therefore, among the mixed reagents listed above, No. 7 and No. 11 are the best and No. 9, No. 6, and those containing ammonium sulfide are the next best. The disadvantage of hydrazine hydrate and hydrazine sulfate is that they cause minute gas bubbles to appear on the slide, especially in and around the test objects. This tendency is somewhat stronger with hydrazine hydrate. Hummel reported that using hydrazine hydrate he succeeded only once in producing hemochromogen crystals from dried powdered blood, but from liquid blood they were easily produced. He speculated that this might be due to gas bubbles.

3. The most serious disadvantage common to ammonium 30/ sulfide and hydrazine reagents is that they either fail to

				14010					
	10% NaOH solution	10% ammonia water	NaOH 5; distilled water 100 hydrazine sulfate 5;	pyridine	ammonium sulfide	10% hydrazine hydrate	saturated hydrazine sulfate	absolute alcohol	distilled water
1 (Donogany)	<u> </u>	_		5.0	5.0	-		_	-
2 (Lochte)	-	-	-	5.0	5.0	-	-	5.0	-
3	2.0	-	-	2.0	5.0	-	-	-	
4	2.0			5.0	5.0		-		5.0
5 (Mita)	-	5.0	-	5.0	-	5.0	-		
6 (Mita)	-	5.0	~	5.0	-	-	5.0	_	-
7 (de Dominicis)	-		10.0	5.0	-	-	-	-	10.0
8	2.0	-		5.0	-	5.0	-	-	10.0
9	2.0	-	-	5.0	-	-	5.0	-	5.0
10	3.0	-	-	3.0	-	10.0	-	-	-
11	3.0	-	-	3.0	-	-	10.0	-	~
12	-	-	-	5.0	-	*	-	-	10.0
13	-	-	-	5.0	-	-	5.0	-	-
14 (Heine)	-	-	-	4.0	-	-	6.0	~	-

Table

* 5.0 of 50% solution

crystallize hemochromogen or crystallize them with difficulty when blood has permeated the tissue to paper or cloth and cannot be separated. Mita stated that when blood had not dried on the surface but had penetrated such materials as linen and filter paper, his hydrazine reagents sometimes failed to produce crystals. In such cases, he recommended, blood should be extracted with glacial acetic acid, and the extract be dried at room temperature to obtain a residue, with which hemochromogen might be crystallized. Lochte also reported that repeated testing with one drop each of absolute alcohol, pyridine, and ammonium sulfide failed to produce the crystals in blood adhered to wool, cotton, or linen fabrics, when intact or teased tissue was treated. He said that blood should be extracted on such occasions with water, ammonia water, 10% soda water, or 10% sodium hydroxide solution, and the residue be used for the crystallization of hemochromogen. Hummel used pyridine and ammonium sulfide and confirmed Lochte's results, but extraction with water as Lochte suggested frequently gave him negative results.

4. My glucose reagents, both the first and the second, are as effective as the best of other reagents in producing the crystals from dried powdered blood. With the glucose reagents the detection of the crystals is easy because the field of view is clear—unlike hydrazine or ammonium sulfide reagents, they do not produce gas bubbles or sulfur crystals.

Although my glucose reagents cannot be proved far superior to other reagents as far as powdered blood is concerned, it becomes clear that they surpass all the others when tests are made on blood that has permeated fabrics or paper and cannot be detached. As Mita, Lochte, and Hummel admitted themselves, their reagents frequently fail to produce the crystals with such test objects, while my glucose reagents easily crystallize hemochromogen. Although the reagents of the following compositions produce hemochromogen crystals fairly well from blood that has permeated fabrics when used in the same way as my glucose reagents, the results are far inferior:

- 1. 10% sodium hydroxide solution 3.0 + pyridine 3.0 + saturated aqueous solution of hydrazine sulfate 10.0.
- 2. (sodium hydroxide 5.0 + distilled water 100.0 + hydrazine sulfate 5.0) 10.0 + pyridine 5.0 + distilled water 10.0.

It is medico-legally very important that blood can be easily 31/ identified from a minute amount of sample without loss of material, and the superiority of my glucose reagents over the others resides in this point. Actual slides are exhibited in the other room; I hope you will look at the proof firsthand.

I must point out, however, that there are some cases in which even my glucose reagents fail to produce hemochromogen crystals. In some cases the spectrum is demonstrated even though crystallization does not take place; in some others, both give negative results. In such cases not only other reagents for hemochromogen crystallization but also reagents for hemin crystallization fail. For example, in 1907 I could demonstrate hemochromogen crystals in the tissues of blue Chinese silk crepe, red cotton cloth, thin red silk, newspaper, and thick Japanese paper that had blood from a human corpse adhering to them; I could also produce the crystals in the part of indigo-dyed cotton cloth that had comparatively abundant amounts of blood, but not at all in parts with a little blood. On July 7, 1907, I demonstrated the spectrum in slides prepared from the plaster and the floor wood of a lecture hall that had been stained with blood of a domesticated rabbit, although I could not produce the crystals of hemochromogen. On the other hand in May, 1908, I failed to produce either the crystals or the spectrum in slides containing blood and powdered iron (1:4) or blood and ash (2:10). Also to be noted is the fact that, when treated with the glucose, hydrazine, or ammonium sulfide reagents, red fabrics sometimes produce red, needle-shaped crystals that resemble hemochromogen crystals; one red dye gives a thick absorption band that corresponds to the first of the two absorption bands of hemochromogen. When test objects are colored fabrics, therefore, control tests using the parts without blood stain must not be neglected. I expect to report on

this subject on another occasion.

Thus far I have talked about dry blood. The method of identifying blood in liquids depends on the nature of the liquids: residue may be tested after evaporation; blood precipitate may be collected according to a certain method; blood pigment may be changed into hemochromogen by adding sodium hydroxide solution to make the liquid strongly alka-

32 line, then dissolving a suitable amount of glucose and pyridine in it, and heating the mixture. In this last method the spectrum is examined, and if precipitate is formed, it is collected using a Spitz glass or a centrifuge at a slow speed and is examined under the microscope, since precipitate sometimes contains hemochromogen crystals.

Lastly, a few words will be added for the clinicians' interest. As early as 1897, Donogany reported that hemochromogen and its crystals could be used for identifying blood in urine, stool, and sputum. Neuberg also mentioned in his recently published Der Harn (The Urine) (Vol. 1, p. 936) the applicability of the hemochromogen crystallization method to the test of blood in urine. I speculate that blood in urine may be precipitated with tannin, zinc acetate, or glacial ethylacetate as O. Schümm has done, collected on a filter paper, and tested, together with the paper if the quantity of blood is small. In case of stool, blood may be extracted with glacial ethylacetate as Über has done, and the residue may be used for hemochromogen crystallization. Blood may then be easily and certainly identified by the presence of the crystals, or the spectrum if the crystals fail to form. How about trying these tests sometime? Our laboratory will supply the reagents.

-Lecture given at a meeting at Kyushu Medical College on May 16, 1912.

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1

On the Behavior of the Coloring Substance of Blood in the Spectrum of Sunlight*

Professor Felix Hoppe

Tübingen

/446 As a result of the experiments of D. Brewster, Herschel, and Muller,[†] the behavior of different dye stuffs with respect to different segments of the spectrum has been determined. In these experiments, it was shown among other things that light of specific refrangibility is so completely absorbed by a large percentage of dyes that, if one lets the rays of the spectrum pass through very diluted solutions of these dyes, dark, rather sharply outlined bands appear in definite places. One can observe the spectrum after the rays have passed through the solution either directly, or by capturing the spectrum on a white surface. At the same time, the experiments showed that from the color of the solutions one may conclude only that these solutions absorb the colors least which they themselves display in white light. On the other hand, one may not conclude from the colors of the dyes in white light which light is absorbed the most.

The absorption bands which appear in the spectrum when it passes through a solution of dye, are apparently characteristics of the dyes, characteristics which often make possible recognition of the dyes when their solutions are very concentrated. They deserve all the more consideration since there is a lack of exact chemical methods for distinguishing dyes and their variable forms.

Pigment such as blood contains displays the same characteristic as do indigo and chlorophyll, dyes which have been previously examined, the characteristic that it can absorb, in a very pronounced fashion, light of a particular refrangibility, and thus produce dark bands in the spectrum which passes through a solution of the pigment. No other red pigment, including chemically altered hematin, displays such bands. This well known combination of apparatus serves the best to test dyed solutions with the spectrum. A heliostat projects the light through a slit into a darkened space—the darkening can be very slight—and onto an achromatic lens. The slit should be at the focal point of the lens. From there, the light is projected into a prism of glass or of carbon disulfide. One now lets the spectrum, produced in this manner, pass through the solution to be tested; this solution

should be placed in a thin container of glass with parallel walls. One may now observe this spectrum either directly with the telescope, or with the unaided eye after intercepting it with a white-paper screen. Hematinometers serve very well as containers for the dye solutions, containers which the optician Schmidt in Berlin has prepared following my direc- 4 tions. In these containers one can test a layer of fluid exactly one centimeter in thickness.

If one now observes a very diluted solution of blood in water in such a container, placed in the spectrum, one will see that, after it has passed through the solution, the spectrum will display two definite dark bands in the yellow and in the green. Both bands are located between the Fraunhofer lines D and E. The band corresponding to the more weakly refracted light is rather close to the double line D; the second band is not so close to E. When the solution is dilute enough, both bands have a width somewhat less than the spectral segment between E and b. If one increases the concentration of the blood solution, or if one lets the spectrum pass through a thicker layer of the solution, the width of both absorption bands increases, but almost exclusively at the cost of the yellow-green light which separates the two lines from one another. When the concentration of the solution is still further increased, the bands finally merge together to form a dark, relatively sharply outlined field. At the same time, more and more of the violet and blue light grows gradually fainter without producing any definite bands. Finally, of the whole spectrum only the segments between E and b, and the red and orange remain. In the case of even stronger concentrations, the green also grows faint and disappears so that only the red with its beautiful Fraunhofer lines remains. I have not pursued the question of the refrangibility of the least red light. While, according to these observations, the blood pigment absorbs with unusual strength the light in the areas indicated between D and E, it leaves intact the sections between A and D as well as between E and b with the same clarity as it absorbs the other sections. The sharpness of the contours of the absorption bands described here results from the fact that the most strongly absorbed sections are closely outlined by the sections most weakly absorbed.

Undissolved blood cells also absorb the parts of the spectrum described here. In order to observe this, it is sufficient to project a spectrum, shining from a prism, by means of the concave mirror of a microscope. (The concave mirror must

^{*} Translation of: "Ueber das Verhalten des Blutfarbstoffes im Spektrum des Sonnenlichtes."

in Archiv für pathologische Anatomie und Physiologie und Klinische Medizin (Virchow's Archiv) 23: 446-449 (1862).

[†] Poggendorf, Ann. d. Phys. u. Chem., Vol. 72, 76 and Pouillet, Müller's Lehrb. d. Physik

be placed so close to the prism that the light which the mirror reflects is parallel or only somewhat convergent). The spectrum must be projected upwards through the opening of the microscope stage onto a thin layer of blood. This layer has been fixed here between the slide and cover slip. If now one removes the tube of the microscope, and looks down perpendicularly at the blood layer, one will recognize most clearly the two absorption bands.

Watery solutions of the blood from the silver-scaled fish, from the *Testudo mauretanica*, as well as from dove, dog, ox, sheep, and pig, all behave in exactly the same manner regarding the absorption bands in the spectrum; these bands are thus to be generally regarded as characteristic of blood from vertebrates.

Arterial blood as well as venous blood shows both of these bands. Lengthy treatment of the blood solutions with carbonic acid does not alter anything. I have also observed that the solution is equally unaffected when the blood is treated with carbon monoxide, hydrogen, hydrogen sulfide, arsenic hydride, nitrous oxide, ether, carbon disulfide, chloroform, caustic ammonia, or arsenic acid. Blood, dissolved in caustic ammonia, still showed the two absorption bands on the following day with undiminished strength. After treatment with hydrogen sulfide, a third band (in the red) appeared in addition to these two bands (a green solution of iron sulfide in a liquid containing ammonium sulfide, such as one would obtain by adding a very diluted iron sulfate solution to ammonium sulfide, does not produce this line in the red).

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Drying the blood at normal temperature does not alter the state of the spectrum.

On the other hand, the absorption lines disappear very rapidly if one adds either acetic acid, tartaric acid, or strong alkalis to the blood solution. The acids work faster than the alkalis in producing this effect. The hematin solution of von Wittich no longer produces the two bands; in sufficient concentration it displays other absorption lines, the stronger of which is found between C and D, close to the last of these two bands. The von Wittich solution corresponds to the blood with regard to the rays of the spectrum which are absorbed the least.

Blood, treated with an excess of cold alcohol, produces a precipitate which, when dissolved in ammonia, no longer displays those absorption bands in the spectrum. Turpentine oil also causes them to disappear. Likewise, the hematin solution, which is obtained by extracting the dried blood with cooking alcohol and sulphuric acid, does not show these bands in the spectrum.

Blood, precipitated with powdery, carbonated potassium hydroxide, retains for days a beautifully arterial coloration, if no heating takes place; if one pours alcohol over the substance, the red coloration soon changes into a dirty brown, and only then does a solution of hematin appear. This solution no longer has the absorption bands. If, on the other hand, one dissolves the moist precipitate in water instead of in alcohol, one obtains a solution which displays both absorption bands just like fresh blood. Likewise, a blood solution

with sodium carbonate will not be changed weeks later with respect to the condition of the spectrum.

In none of the fluids which failed to display the absorption bands could these be reconstituted by treatment with alkalies, etc.

If one precipitates the blood solution with an excess of lead acetate, filters it, and then, by using sodium carbonate, precipitates the lead from the filtrate, one obtains a solution which produces most sharply the absorption bands in the spectrum.

If one induces hematuria by injecting gallic acid salts into the veins of dogs, the urine does not display the absorption bands in the spectrum, although one is able to produce hematin from such urine; this urine also does not turn red on contact with oxygen.[†]

From the behavior of unaltered blood, as well as from that of blood treated with various reagents, it turns out that the contents of the blood cells (the serum displays no noticeable absorption in the yellow and green, when the layer of the serum is not thicker than one decimeter) very strongly absorb the indicated parts of the spectrum, as long as the 449. albumin substances of this liquid have not coagulated or been transformed into alkali or acid albumin. Now, since a substance which shows such definite absorption cannot appear colorless as do the well known albumin substances, one would thus have to assume that this very substance, which gives to the contents of the blood cells its red color, also produces this absorption. Further, since this absorption capacity appears to be independent of the most varied color alterations which the blood undergoes when treated with oxygen, carbonic acid, carbon monoxide, arsenic hydride, etc., and, on the other hand, since this absorption capacity is destroyed by relatively weak processes which, however, affect coagulation or the altering of all albumin substances, the following assumption seems to be justified. All those changes, which produce the gases described in blood pigment, do not destroy the pigment. Moreover, one may now hope to find a means by which altered blood can be transformed again into normal blood.

As a result of the reactions mentioned above, it also appears certain that there is a compound in the blood cells which produces the pigment of blood, and causes these absorption bands. It is not precipitated by lead acetate; it dissolves much more easily than albumin; and, when acted upon by acids, caustic alkalis, etc., it breaks down into an albumin substance and hematin, which is contained in the von Wittich solution. Without a doubt, this is the very body which forms the Funk crystals. If this representation is correct, then it follows that the attempt to obtain uncolored blood crystals is in vain, although it might be possible that, when the corpuscle breaks down, substances can form which are equally capable of crystallization. I am now carrying out the purification and chemical testing of this "blood red".

For the forensic identification of blood in stains on cloth-

[†] One can conclude from this that, in the kidneys, the transuded blood pigment is probably decomposed by a secreted acid.

ing, etc., one already possesses a rather large number of test transparent paper, must be somewhat moistened and then stains, which are not extracted, and are on white linen or between the prism and the eye.

methods which are in part exact. One can naturally also reveal, in a sunlight spectrum, the bands described, when make use of the testing method presented above. Blood- they are placed so as to intercept the rays of the spectrum

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Tincture of Guaiacum (Guaiacum Officinale L.) and an Oxone Vehicle as Reagent for Very Small Amounts of Blood, Specifically in Cases in Forensic Medicine*

J. van Deen

7228 The smallest blood volume of any age, even with a substantial admixture of other substances, stains blue when tincture of guaiacum and an ozone vehicle (for example: oil of turpentine) are added.

The following experiments are intended to confirm the above:

1. A minimum amount of aged, fetid blood, which had been stored for approximately 8 to 9 months, was diluted with distilled water until the fluid became nearly achromatic. When a few drops of this mixture were added to oil of turpentine, which has a high ozone content, intensive blue staining soon became evident.

The blue staining was also definitely determinable when the blood was initially left standing for 24 hours with oil of turpentine and tincture of guaiacum was subsequently added. When the blood, mixed with oil of turpentine, is filtered, the tincture of guaiacum had no effect on the filtrate, probably because the turpentine did not pass through the filter, and its ozone content was not absorbed by the blood, prior to the admixture of the tincture of guaiacum.

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2. Blood was left standing for two years with glacial acetic acid and was diluted in minimal parts with water until the fluid appeared nearly achromatic; then a few drops of oil of turpentine and tincture of guaiacum were added. The blue staining developed immediately.

3. When more glacial acetic acid was added to the blood mentioned under 2, followed by filtration, a minimum of the filtrate still stained blue with oil of turpentine and tincture of guaiacum. However, in some cases the staining soon vanished. Boiling did not inhibit the blood reaction.

4. Minimal quantities of blood which had been stored in alcohol for two years and which contained numerous coagulated particles were treated with oil of turpentine and tincture of guaiacum; blue staining developed immediately. The blue staining could not originate from the alcohol; it could, however, originate from the solid particles in the same, despite their microscopically small size.

5. A three-year old desiccated calf blood clot was finely pulverized; 0.1 g of the same was mixed with 400 g water and repeatedly agitated. A few drops of the above mixture soon

stained blue with oil of turpentine and tincture of guaiacum.

6. A drop of the mixture mentioned under 5, mixed with five drops of water still reacted, even when 1/6th of the mixture was subjected to the procedure.

7. When the last-mentioned dilution was doubled, so that 1 drop contained not more than 1:40,000, the reaction still took place.

When very small considerably diluted blood volumes are used, the start of the reaction will take place after a few moments' delay only.

Since Schoenbein demonstrated that the iron in the blood 230/ probably transfers the ozone from the ozone vehicle to the tincture of guaiacum, control tests with iron preparations had to be performed.

Ferric oxide, ferric hydroxide, caput mortuum and ferrous carbonate were tested with negative results.

Moreover, it was found that iron filings, ferrous oxide, hydroferrocyanic acid, calcium ferrocyanide and ferric phosphate do not act as ozone transmitters. However, the following act as such:

a) to a moderate extent: ferrous sulfate, ferric lactate, ferrous iodide and ferrous sulfide;

b) to a significant extent: ferrous acetate, ferrous citrate and ferric chloride, in particular the last-mentioned salt, which is no less effective in this respect than old, fetid blood.

CuSO₄ and cuprous acetate were recognized as ozone transmitters as well, but not to a very great extent.

Various preparations, in particular the red lead and antimony preparations, yielded negative results. The same applies to red dyes such as logwood, brazil wood, sandalwood and carmine. None of the iron preparations which act as ozone vehicles has a color similar to the color of blood; mistakes therefore do not occur readily. Ferric acetate is red, but its color is of a much brighter shade than the color of blood. Furthermore, it is easy to determine with ammonia water whether the investigated substance is an iron preparation or blood. When tested with the above-mentioned substance, iron preparations show significant clouding which soon deposits in the form of a red precipitate. Blood, on the other hand, will show a greenish-yellow discoloration. A minimum of iron content results in a yellow solution, but the substance remains clear, while fluids containing blood are never transparent. Not too much ammonia water should be used for the test.

Translation of: "Tinctura Guajaci und ein Ozonträger, als Reagens auf sehr geringe Blutmengen, namentlich in medico-forensischen Fällen".
 in Archiv für die holländischen Beiträge zur Natur- und Heilkunde 3: 228-231 (1862).

Identification of Blood

The difference, as compared with the blood reaction, /231 specifically the fresh blood reaction, was less evident only in case of ferrous sulfate. But the difference nevertheless became determinable a few hours later, and especially after a few days. Potassium ferrocyanide prevents mistakes, i.e.,

mistaking a blood reaction for a copper reaction.

Aged blood which has become fetid, especially when stored in fluid condition, has a stronger capacity as ozone vehicle than fresh blood.

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The Reaction of Certain Organic Compounds with Blood, With Particular Reference to Blood Identification*

Oscar Adler and Rudolf Adler

(Received by the Editorial Offices on January 7, 1904).

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- Among methods used for blood identification, the preparation of hemin crystals is to be considered primary; under certain circumstances, albumin and iron determination can be significant as well for blood verification. Clinically, the guaiacum test is widely used for blood determination in urine, in gastric juice and in feces. Recently Vitali¹ spoke warmly in favor of the test. As known, the guaiacum test is based on the fact that hemoglobin can transfer oxygen originating from turpentine oil or from hydrogen peroxide into the active ingredient of guaiac resin, named guaiaconic acid by Hadelich; a neutral substance, or a substance which stains blue in acid solution will result, and is named a guaiaconic acid ozonide.

We used guaiacin successfully for blood identification instead of guaiacum tincture. The substance, prepared by Schmitt² from guaiacum wood, proved to be more sensitive. We mixed the fluid to be analyzed with a small volume of hydrogen peroxide and covered the mixture with a layer of an alcoholic guaiacin solution.

More recently Rossel³ recommended Barbados-aloin for blood determination in urine. According to Utz⁴, the test is less sensitive than the guaiacum test.

760 The purpose of present study is the systematic investigation of numerous chemical compounds which show chromatic reaction as a result of oxidation, in the presence of blood (when hydrogen peroxide is added). Some of these substances were formerly used for the verification of ozone⁵ and oxidizing ferments (oxidases)⁶. We considered the sensitivity of the reactions, as well as the behavior of control reactions; our results could, therefore, perhaps be of interest for the study of oxidizing ferments as well.

11.

We report the result of our systematic investigations below. A large number of substances is to be considered in this context; we limited ourselves to the aromatic amidosubstances, phenols, aromatic acids and to the diphenyl- and naphthalene groups⁷. We studied the behavior of the reduction products of certain tar dyes (leucobases) in conjunction with the above.

The blood used for the experiments was collected from the carotid of the animal (rabbit), immediately defibrinated, and a specific concentration was obtained for investigation purposes by diluting the blood with distilled water. The reaction took place as follows: at the start, the desired blood concentration of 0.001% (i.e. a 100,000-fold dilution of the blood) was obtained; then hydrogen peroxide was added and the test continued until a definite chromatic reaction occurred, or until a chromatic difference as compared with the control became evident. As the following table shows, we define this point of the test as the sensitivity limit of the reaction.

The following is added for the clarification of the Table: all reactions were performed with the aid of controls (identical testing conditions, but without the presence of blood). When the control showed no chromatic modification, the result was defined as negative. But whenever the control reagent showed a chromatic change due to atmospheric oxygen, only the color difference could, of course, be taken into consideration.

III.

As the Table above indicates, the reactions of the individ- 63/ ual representatives of the listed groups (amido-substances, phenols, acids) vary during the described procedure, i.e., the higher members of the series generally proved to be more sensitive.

In conjunction with these experiments, we investigated some readily oxidizable leuco-bases of the triphenylmethane series. We found that the malachite green group (malachite green, brilliant green, acid-green) and the pink aniline derivatives (dahlia, methyl violet, crystal violet) are preferable for the above-mentioned purpose. The rest of the triphenylmethane dyes (alkali blue, ketone blue, patent blue, cyanin, Turkey blue) and the eosins and rhodamines do not yield satisfactory results.

We wish to convey thanks here to the Dye Works (formerly Meister, Lucius and Brünning) for making many of their products available.

In view of the fact that all above mentioned reactions occur because the blood pigment transfers oxygen originat-

^{*} Translation of: "Über das Verhalten gewisser organischer Verbindungen gegenüber Blut mit besonderer Berücksichtingung des Nachweises von Blut".

in Hoppe-Seyler's Zeitschrift für Physiologische Chemie 41: 59-67 (1904).

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Identification of Blood

Substance	Reaction of the Solution	Color Reaction	Sensitivity Limit (%) Blood Solution	Control
A. Amido-substances				
) monamines				
aniline	acid (HCl, H_2SO_4)	black-green	0.1	negative
monomethylaniline	acid (HCl)	dirty violet	0.1	"
dimethylaniline	acid (HCl)	light yellow	0.1	17
diphenylaniline	acid (acetic)	green	-	1/
p-toluidine	acid (HCl)	red	0.01	"
xylidine	acid (HCl)	brown-red	little sensitivity	17
b) diamines				
o-phenylenediamine	neutral	brown	0.007	discoloration after
m-phenylenediamine	neutral	violet	0.007	
				standing for some
p-phenylenediamine	neutral	brown	0.007	time.
dimethyl-p-phenyldiamine	neutral	red	0.009	
tetramethyl-p-phenylene-			>	early color
diamine	neutral	violet	0.009 🏓	balance
B. Phenols				
			•	
a) monohydric phenols			44	
phenol [*]	neutral or alkaline	red brown	little sensitivity	negative
	(NaOH)			
p-amidophenol	alkaline (NaOH or	violet	0.008	early color balance
	Na_2CO_3)			
o-cresol*	neutral or alkaline	red brown	little sensitivity	negative
	(NaOH)			
m-cresol*	neutral or alkaline	red brown	" "	"
in-cressi	(NaOH)	ica biowii		
	· · · · /	, ,	. .	. "
p-cresol*	neutral or alkaline	brown red		- //
	(NaOH)			
thymol	alklaline (NaOH or	brown red	" "	17
	Na_2CO_3)			
b) dihydric phenols				
pyrocatechin	alkaline (NaOH)	green	0.005	color balance develops
F)		0		after some standing
guaiacol†	"	yellow brown	0.05	negative
•	"	•		
resorcinol	"	greenish	$\left\{\begin{array}{c} 0.01\\ 0.00\end{array}\right\}$	color balance develops
hydroquinone		brown yellow	0.005	after some standing
orcin (methyl	"	red	0.002	color balance develops
resorcinol)				after prolonged standin
c) trihydric phenols				
pyrogallol	"	brown	0.005	early color balance
phloroglucinol	"	violet	0.005	"
· •			0.000	
C. Aromatic acids				
benzoic acid	alkaline (NaOH)	_		
salicylic acid	17	brown	very little	negative
2			sensitivity	0
pyrocatechuic acid	"	pink (vanishes	0.001	negative (yellowish)
pyrocateenale acid			0.001	hegative (yenowish)
	"	gradually	0.005	
gallic acid	"	brown	0.005	color balance
Diphenyl Group				
benzidine [*]	acid (acetic)	green [§]	0.001	negative
tolidine [†]	"	red	0.05	*
		green) 0.25	"
Napthalene Group			-	
α-naphthol	alkaline (NaOH)	brown	not	negative
· ·			(" "
β -naphthol		brown yellow	∫ tested	"
α -naphthylamine†	acid (HCl)	dirty blue	little	~
			sensitivity	

* alcoholic solution * behavior of creosote from Buchholz tar is, of course, similar i stains blue after prolonged period of time

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ing from hydrogen peroxide to the respective oxidizable substances, the logical conclusion is reached that other substances can trigger analogous reactions as well.

Among the substances to be considered we mention the following here: iron-oxide salts (Vitali⁸), the thiocyanate salts (Tarugi⁹), certain oxidizing ferments (indirect oxidases¹⁰); all these substances are able to act as indirect oxidizing agents in the presence of hydrogen peroxide. In animal fluids containing leukocytes (urine, saliva, pus) oxidizing ferments which are destroyed by boiling are presumably present. As a matter of course, pus containing blood, as usually found in medical practice, reacts after boiling as well. No conclusive experimental studies are available as of now concerning the behavior of pus without any

even minimal trace of blood. In contrast to the substances mentioned earlier, other reducing substances can have a disruptive effect. Uric acid, for example, can inhibit the sensitivity of the leuco-malachite test (see below); but such an effect can be eliminated by proceeding according to Weber's guaiacum test (see below). Finally, it should be pointed out that substances which trigger a secondary reaction (iron salts: yellow staining of malachite green; nitric acid: formation of diazo-substances) should be taken into due consideration.

Summarizing test results obtained as of now, we feel justified in saying that even minimal blood traces (dilution: 100,000-fold) are determinable with some of the abovementioned reactions. Therefore, whenever test results are negative (see below), it is reasonable to assume that no blood is present.

A few suggestions for the practical utilization of the blood tests described are listed below.

IV.

Blood Identification

The chemical identification of blood is of foremost importance, chemically as well as forensically. Accordingly, efforts were made for a long period of time to devise methods for blood verification even when the amount of blood is minimal. However, besides the importance of positive blood identification, definite proof that no blood, not even minimal quantities of it, is present, can—under certain circumstances—be of great significance as well.

It is known that the negative outcome of one of our finest methods: the preparation of hemin crystals, does, under certain circumstances, not represent definite proof of the ab-

65 sence of blood. Cases are known when the presence of blood was verified despite the negative outcome of the Teichmann test.

We list a few tests below, intended for the identification of blood stains, the identification of blood in water, urine and in feces.

Identification of blood stains. We used leucomalachite green (leuco-base of malachite green) for this purpose. The test was performed as follows: the stain to be investigated is

thoroughly soaked with the reagent (see below); then a 3% solution of hydrogen peroxide is poured over the stain. If it is a blood stain, the stain will immediately turn green. The reaction is also well-defined when minimal, hardly perceptible, blood traces are present. The reaction will also occur with boiled blood stains.

We preferred to prepare the reagent as follows: a concentrated solution of completely achromatic, chemically pure, leuco-base of malachite green (tetramethyldiamidotriphenyl-methane)¹¹ is prepared in glacial acetic acid. A minimal green color will develop in most cases even when a completely achromatic preparation is used; the green color is eliminated by adding an equal volume of chloroform. Water is then added drop by drop while the mixture is carefully agitated until the chloroform precipitates entirely. The green chloroform is then separated from the supernatant reagent. The eventual clouding of the reagent, which can be caused by the precipitation of the leuco-base, is eliminated by adding glacial acetic acid. If traces of green color are still evident in the reagent, these are removed by shaking the reagent with a small quantity of chloroform. The reagent, prepared as indicated, should be entirely achromatic.

We pointed out earlier that substances other than blood can cause a positive result of the test. The possible presence of such substances should therefore be taken into consideration. I refer in this context to statements made in Part III, page 63 and following pages.

As for the negative outcome of the test: we found that the presence of iron salts in ample quantities can prevent green staining, even when blood is present; yellow staining will 66/ develop instead. Irons salts should therefore be excluded (iron tests).

When all precautions are taken into consideration, the negative outcome of our test leads to the conclusion that not even minimal traces of blood are present.

Identification of blood in water

Due to their high sensitivity and due to the completely negative outcome of the control tests, the following substances are suggested for blood identification in water: leucomalachite green (see reagent preparation), crystal violet leucobase and benzidine.

For the benzidine test we used alcoholic benzidine solution, concentrated while heated and filtered after cooling.

For the implementation of the test, we mixed the water to be investigated with a small volume of hydrogen peroxide and a few drops of acetic acid; then a few cubic centimeters of the benzidine solution were added. A spendid green staining develops when blood is present.

The test succeeds also when performed after boiling the water containing blood.

Regarding the precautions, we refer to Part III, page 63 and following pages.

The high sensitivity of the leuco-malachite green test and of the benzidine best is significant, as compared with the spectroscopic method and the Teichmann test. The Teichmann crystals can be obtained when the dilution is at least 20,000-fold.¹² Moreover, blue staining develops in the guiaiacum test in a 500-fold dilution.¹³ On the other hand, the leuco-malachite green test and the benzidine test show definite color reactions even in a 100,000-fold blood dilution.

Blood identification in urine and feces

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- *Urine:* the aforementioned tests with leuco-malachite green and benzidine are suitable for blood identification in urine as well, when proceeding according to Weber's modification of the guaiacum test.

10 to 15 cc urine is mixed with half that volume of glacial acetic acid, whereby hemoglobin is converted into hematin. Agitation with ether follows, and the hematin mixes with the ether. If the ether in the above mixture forms an emulsion, the liquid can be separated by adding a few drops of alcohol. The ether is removed; then the leuco-malachite green and a small volume of hydrogen peroxide is added. Should some of the leucobase precipitate during this process, the precipitate can be dissolved by adding a small amount of glacial acetic acid.

Instead of the leuco-malachite green reagent, the following can be added to the ether: an alcoholic benzidine solution, some hydrogen peroxide and a few drops of acetic acid.

When the urine contains blood, the color reactions mentioned earlier will develop. Feces: A small quantity of the feces to be investigated is slightly diluted with water. Then 3 cc of the diluted and unfiltered feces is mixed with 2 cc of the aforementioned benzidine solution and with 2 cc hydrogen peroxide (3%); a few drops of acetic acid are added. Intensive green staining develops when blood is present.

Notes

- 1. Vitali: Giorn. di Farmac. di Trieste, 1902, vol. 7, p. 193
- 2. Schmitt: Le Bois de Gajac, Thesis at Nancy, 1875, and Mercks Bericht. 1902, p. 75.
- 3. Rossel: Schweizer Wochenschr. Chem. Parm., 1901, No. 39, p. 557ff.
- 4. Utz, Oesterreich. Chem. Ztg., 1902, vol. 5, p. 558.
- See C. Arnold & C. Mentzel: Ber. Dtsch. Chem. Ges., 1902, 35: 1324 and 2902; Chlopin: Ztschr. f. Untersuch. v. Nahrungs- und Genussm., 1902, 5: 504
- 6. See Neumann-Wender: Chem. Ztg., 1902, 26: 1217 and 1221
- 7. Thallium hydroxide also indicates the presence of blood under similar conditions. We have not considered the other inorganic compounds.
- 8. Vitali: Giorn. di Farmac. di Trieste, 1902, vol. 7, p. 193.
- 9. Tarugi: Gazz. Chim. Ital., 1902, 32. 1 vol. 505.
- 10. See Bourquelot: Congr. Pharm., Paris 1899, Répert. Pharm. and Neumann-Wender: Chem. Z1g., 1902, 26: 1217 and 1221ff
- 11. A leucobase especially prepared for this purpose can be obtained from the Chemistry Laboratory of the wholesale pharmacy Wilhelm Adler at Karlsbad, where the prepared reagent is also available.

- 12. Hager: Handbuch der Pharm. Praxis, vol. II, p. 879.
- 13. Hager: as cited above, p. 881

The Chemiluminescence of Hemin: An Aid for Finding and Recognizing Blood Stains Important for Forensic Purposes * ¹

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- In the unraveling of capital crimes, the discovery and proper evaluation of a blood stain, the identification of blood as such, as well as the determination of the blood type often play an important role, indeed frequently a deciding one.
 Blood stains are very different, depending on the type of injury involved, and on the kind of material to which the stains adhere. For the expert who thinks and works compre
 - hensively, in accordance with the principles of criminalistics, the proper evaluation of the blood stains present will always be of the greatest importance. From the general type and the direction of the blood spatters, and from the place where the stains were discovered, it is often possible to reconstruct essential details of the crime.

The presence of even the smallest blood spatters at the scene of the crime, on the clothing of the assailant, or on the criminal himself can be of decisive importance. It is, however, not always easy to identify blood traces at the scene of the crime, or on the weapon, especially in those cases which involve an old weapon or old blood stains.

Temperature, substratum, sun rays, moisture, as well as artificial washing, and chemical changes of the hemoglobin can fundamentally alter the external state and color of blood stains. Frequently, blood stains are covered over with dirt smudges which makes it difficult to recognize them. On the other hand, sometimes the dried remains of red fruit juices, for example, or of tobacco saliva, fungus, and mildew, are not unlike blood stains.

While *fresh blood traces* can naturally be identified with ease, usually by the microscopic identification of blood corpuscles, to identify *older blood stains* always requires special aids.

The hematin, which has been formed in old, dried blood demonstrates the well-known dichroism which simplifies the recognition of blood stains. Especially in the sunlight, an

older blood stain glimmers with a green hue in reflected light, but with a reddish color in direct and indirect light. Blood stains, however, do not always lie exposed to the light of day. There are, accordingly, limits set in recognizing a blood spot by means of the optical appearance of dichroism. For example, the blood could have been removed from the substratum by cleaning, while it might still be present in the smallest portions in the dust in floorboard cracks, in the seams of shoes or in the seams or hems of a suit, or washed through to the suit lining; it could also be lodged in the smallest openings or notches of a tool or some similar instrument. Not infrequently, one must carry out a test for blood traces in wash water or the remains of liquids caught in goose-neck pipes in the relevant drains or wash basins.

Blood traces outside can become unrecognizable to the naked eye within a short span of time as a result of meteorological or mechanical conditions, or, among other causes, as a result of frequent downpours. One must resort to preliminary chemical tests to discover such hidden blood traces. 227. These tests are all based on the catalytic effect of blood, the effect of transferring oxygen. One thinks of the hydrogen peroxide, tincture of guaiacum, and benzidine tests. A positive result of these tests is, however, not conclusive proof of the presence of blood. Moreover, carrying out these tests has the disadvantage that some of the material is lost.

The hydrogen peroxide reaction must be characterized as very dangerous, especially since steel rust, which unpracticed observers can confuse with blood traces, produces a catalytic decomposition of hydrogen peroxide.

On the other hand, the positive results of microchemical reactions, by means of which the characteristic crystals of hemoglobin and its derivatives (*Teichmann* hemin and hemochromogen crystals) are produced, are proof of the presence of blood. The *Teichmann* crystal test, however, is bound up with a number of difficulties, in so far as the crystalization does not take place when insignificant mistakes have occurred in carrying out the reaction, or when different chemicals are present. Moreover, this test gives a negative result when the solubility of the hemoglobin is reduced.

The identification of blood can only be considered as cer-

^{*} Translation of: "Die Chemiluminescenz des Hämins, ein Hilfsmittel zur Auffindung und Erkennung forensisch wichtiger Blutspuren."

in Deutsche Zeitschrift für die Gesamte Gerichtliche Medizin 28: 225-234 (1937).

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tain if one of the characteristic blood absorption spectra is obtained from a suspected stain. For a long time it was only possible to link the hemochromogen test directly to the spectroscopic test. After carrying out the other preliminary chemical tests, the use of spectroscopy is no longer possible. According to K. Gleu and K. Pfannstiel², when 3-aminophthalhydrazide in alkaline soda solution and hydrogen peroxide or diluted sodium peroxide solution is added to hemin, an intensive chemiluminescence takes place. In pursuing a suggestion made by these authors, we tested to what extent and with what results this light reaction was useful in discovering and characterizing blood traces of forensic significance.

Proceeding from the model test with crystalized hemin (Figure 1)³ [figures not reproduced in the translation], we thoroughly tested fresh and old blood traces for their behavior with the test solution. As part of the wide ranging experiment, such stains were included which could have simulated

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used to carry out the experiments. The composition of the solutions is as follows. 1. About 1/10 g luminous substance, 5 g calcium carbonate, 15 cc 30% hydrogen peroxide in 100 cc of distilled water.

the presence of a blood trace. Two reaction solutions were

2. 0.1 g luminous substance in 100 cc 0.5% aqueous sodium peroxide solution.

Both solutions are usable for the experiments and are, in themselves, free of any luminescence in accordance with the requirements—the distilled water must be free of oxidizing agents such as hypochlorite.

In the course of the experiments, we found that it was expedient to alter solution No. 1—should this one glow lightly contrary to our expectations—with a trace of "indazolon-4carbonsäure" in order to obtain a completely problem free, non-glowing reaction solution.

Both test solutions have shown no differences in their reactive capability, so that in practice one can work as well with one as with the other.

Fresh blood at times induces only a weak glow. Dried blood traces, however, call forth a bright, blue, long-lasting chemiluminescence when they come into contact with the test solution. The older the blood trace was, the more clearly the light effect showed up. This was caused by the hematin in the blood which had separated from the globin in the course of aging.

7229 The objects to be tested for the presence of blood were sprayed with the solution, which at first did not glow. It is an advantage if one uses for this a glass sprayer. The many tests⁴ which have been carried out demonstrated that even the smallest blood traces produce a strong luminosity. The identification of blood traces by means of chemiluminescence can be characterized as specific, since sperm, saliva, urine, excrement, pus, and other body fluids do not react in this fashion. Milk and coffee stains as well as starch, reddyed jellies, inorganic and organic dyes (such as eosin, Sudan red, scarlet red, and others), carpets, tissues, leather, skin, fungus cultures of the most varied types, moldy bread,

oils (oil paints, varnish, mineral oil), and colored waxes do not induce a glowing reaction when blood is not present. Samples of earth (humus, clay, sand), stone, wood, and metal samples (copper, steel, brass, lead, zinc, and others), grass, and foliage, fail by themselves to demonstrate luminosity. In particular, rust and other metal oxides, which occur frequently in practice, and which are not infrequently encountered together with bloodstains, and have a strong dissociating effect on hydrogen peroxide, could not stimulate the luminous substance to give a blue luminosity.

In accordance with these findings, it was to be expected that blood traces on the greatest variety of substrata would reveal themselves through the luminescence.

The experiments carried out along these lines completely ² confirmed this supposition. A few photographs show most clearly the findings of the test.

One can see in figures 2 to 5 [not reproduced in the translation] that, at any given time, only the portions of the substratum which were moistened with blood are illuminated. In the comparison pictures, which were taken of the test objects by daylight, the places moistened with blood are from time to time outlined.

Even traces, which have been washed thoroughly by long 2 rain (see Figure 3), and other similar traces on foliage, grass, earth, and stone, which are not visible to the naked eye, glowed with undiminished intensity after being sprayed with the test solution. Other, even longer-lasting effects were observed. After a successful reaction, the glowing phenomenon could be induced again by repeated spraying with the test solution.

Likewise, it was possible using chemiluminescence to identify small blood traces in large quantities of water, in soapy water, and in other waste water.

Thus, the presence of blood in liquids can certainly be 2 identified, namely in:

- (a) 2 drops blood in 1ℓ water pipe water
- (b) 4 drops blood in 2l soapy water
- (c) 6 drops blood in 6l soapy water

(d) 6 drops blood in 5l dirty water.

In the case of very dirty water, the luminescence appears more clearly only after the suspended particles have settled. One ought to notice that, naturally, the strength of the glowing effect in liquids of very slight blood content is lessened. By adding a trace of caustic lye, however, the glowing capability of the solutions is raised, though the duration of the effect is lessened. A glowing reaction of a minute's duration is the standard for judging the experiment. One must, at this point, also take into consideration that the hypochlorite of tap water or of soap can induce a very weak, but perceivable, luminescence even though it lasts only a second and very rapidly dies away. Figure 6 reproduces the photograph of the test.

Finally, we should mention that blood traces on the hands can likewise be made visible by spraying with the test solution, as Figure 7 demonstrates.

This new blood identification is all the more useful for the

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forensic chemist since spectroscopic as well as serological testing and identification of the blood type are still possible after this testing of the materials. After a successful glowing reaction, the extractions prepared from the test objects with physiological saline solution produced clear absorption spectra in every case.

After the duration of action of the test solution on the blood stain, the spectrum of the alkaline or neutral methemoglobin was established. Further, it was possible to obtain the hemochromogen spectrum by adding pyridine and sodium hydrosulfite to the blood solution. Even those blood traces which were no longer perceptible to the naked eye, but were discovered by means of chemiluminescence, were successfully submitted to spectroscopic analysis. Likewise, the Uhlenhuth precipitation reaction could be carried out with the traces of blood. From blood mixtures, the individual blood types could be recognized in the usual way. Blood traces whose type was unknown could be positively analysed in the same fashion. Difficulties in the serological differentiation of blood mixtures were not noticed. The precipitations were successful even when the test solution had dried on the blood trace.

Summary. Chemiluminescence of 3-aminophthalhydrazide in soda alkali solution is released with exceptional strength in the presence of small amounts of hydrogen peroxide by means of hemin, but also gives considerable luminosity with dried blood.

The luminosity reaction can thus be applied to forensic practice with success. Even blood traces which are a year old, or even older, excited the test substance to luminosity.

Strong oxidizing agents, such as hypochlorite, ferricyanide manganese dioxide, colloidal platinum, osmium tetroxide, and gold chloride, are also capable of producing a weak luminosity in the 3-aminophthalhydrazide by means of a catalytic dissolution of hydrogen peroxide. The luminous effect, however, is far stronger in the case of the action of dried blood, which as a catalyzer, is able most strongly to activate the peroxide of the test solution. In addition, in forensic practice when dealing with samples used for conviction one need scarcely reckon with the presence of the catalyzers of an inorganic nature, mentioned above. The materials of everyday life, which might simulate a blood trace, demonstrated no luminous reactions under the test conditions given. Far exceeding the significance of the luminous reaction as a preliminary test for the presence of blood is the fact that one can-and here especially lies the worth

of the new blood reaction—carry over the blood trace, unaltered by its discovery through luminescence, to the pretreatment processes necessary for characterization, i.e., the spectroscopic and serological examinations.

A further advantage of the experimental method de-234, scribed here, as opposed to those presently employed, is that, even an extended area where the crime occurred, or a larger piece of evidence, can rapidly be tested thoroughly for the possible presence of blood stains without wasting supplies.

Finally, we should mention that the luminescence of blood traces appears with special clarity in the dark. The intense, uniform, blue light permits fixing the position of blood spots photographically without any further equipment.

For the accompanying photographs of luminescence in the open air, the exposure time amounted to five minutes; for the example experiment (Figure 1) a five to six-hour exposure was sufficient.

We will report at some time in the future to what extent blood traces are still suited for determination of blood groups and factors, after having undergone the luminescence reaction.

Notes

- 1. Lecture given at the meeting of the Deutsche Gesellschaft für Gerichtliche und Soziale Medizin, September, 1936, in Dresden.
- 2. I refer to the fundamental works of Gleu and Pfannstiel, "Benzisoxalon-4-carbonsaure und Indazolon-4-carbonsaure" and "Über 3-Aminophthalsäurehydrazid" in J. prakt. Chem. NF 146, 129, 137 (1936). These works give the particulars on pure preparation of 3-aminophthalhydrazide, the so-called white hydrazide, as a luminous substance, the proportions of isomers of the hydrazide, and the procedure for the luminosity reaction.
- 3. This print was kindly placed at my disposal by Mr. Pfannstiel.
- I thank my coworkers in the institute, Mr. Koch and Mr. Ahlendorf, for their helpful assistance in carrying out the experiments.

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- 2. Höber, R. Lehrbuch der Physiologie des Menschen. 4 Aufl. Berlin: Julius Springer.
- 3. Jeserich, R. Chemie und Photographie im Dienste der Verbrechensaufklärung. Berlin: Verl. Stilke 1930.
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