

## **Section 2. Identification of Body Fluids**

Orfila's paper on examination of seminal stains is one of the oldest papers on this subject in the literature. A number of chemical tests were proposed, and microscopical examination for the identification of spermatozoa was rejected because of the poor results. As is made clear in Bayard's paper, Orfila's results were unsatisfactory in part because of the technique used to isolate the cells, and perhaps in part, too, because the microscopes were not that advanced. Bayard's paper, a classic, placed the priority of microscopical examination of seminal stains on a firm footing. Various chemical reactions continued to be used for a long time, though, as is clear from Lassaigne's paper. Lassaigne (1880-1859) was quite well known. Paul Brouardel's paper tends to stress microscopy. Brouardel (1837-1906) held the Chair of Legal Medicine at Paris, and was Dean of the Faculty of Medicine there as well. Cauvet's paper details how a sexual assault case was analyzed at the time in terms of both blood

and semen. Brouardel and Boutmy's report was an assessment of nonmorphological dyeing technique for the identification of seminal stains. Charles Robin (introduced in Section 1) and his colleague Ambroise Tardieu (1818-1879) discussed a number of less frequently encountered body fluids in their paper.

Florence's papers discuss in great detail the history of medico-legal examinations in sexual assault cases. He gave a good deal of information about the detection and identification of spermatozoa as well. In the papers, he introduced the now well known Florence crystal test for seminal stains. Florence (1851-1927) spent most of his professional career at Lyon. Barberio introduced another crystal test for seminal stains which enjoyed some popularity as well.

Lundquist's paper in 1945 introduced the acid phosphatase test for medico-legal detection of seminal stains. This test is in very wide use today.

## Semen Considered from a Medico-legal Viewpoint\*

M. J. B. Orfila

469 I have often been consulted by magistrates, to find out if stains present on linen were formed by semen, fat or matter from discharge of venereal disease, from leukorrhoea, etc. Other physicians have been required by courts to give their opinion on similar questions; science, however, possesses no specific ways of facilitating the solution to this problem. This consideration would have been sufficient to involve me in publishing a few experiments I had attempted on this subject, if I had not been provoked into it by reading a report prepared a few months ago by Dr. X . . . in an affair of child molesting. Called upon to confirm the condition of the sexual parts of a young girl of thirteen years and nine months, who was believed to have been violated *nine days* beforehand, this physician concluded that the act of copulation had been consummated, supported, among other facts, by his withdrawal of a *certain quantity of semen from the vagina*. In a consultation asked of me, I was asked: is it possible to allow that semen was found in the vagina of the girl R . . . whose examination only took place nine days after the alleged consummation of the act? It is all the more implausible as, this girl having a mucus discharge, the semen would have been carried out by the matter of the discharge. Besides, how can one be sure that liquid drawn from the vagina was semen rather than mucus? What attempts were made to solve this question? Why not resort to chemical experiments, to examination by microscope? It is necessary to remark in the interest of truth, I added, the author of the assertion concerned did not sufficiently appraise its value before announcing it; he would have seen he could be compromising his reputation in deciding a question of this importance with such levity. The accused was acquitted.

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Here now is the procedure I followed in this research: I examined comparatively linen stained by semen coming from several individuals who had had nocturnal emissions, and others who had been hanged, in whom there had been ejaculation. I then studied with several repetitions, on several subjects, the characteristics of stains made on linen by the substance of vaginal discharge in acute and chronic leukorrhoea in young girls and adults; and in venereal disease in women incontestably presenting symptoms of syphilis. I also submitted to my examination substance from a discharge of the urethral canal in a case of an internal one-eyed fistula, the sequel of several external fistulae from gonorrhoea, five days after cauterization. Finally, to complete this work, I

wanted to find out the behavior of linen stained by the matter of whitish lochia, incorrectly called milky, as well as by fat, by saliva and by nasal mucus. It seemed to me that, in establishing the differences between the various liquids and semen, I can consider the problem with which I am occupied as resolved.

**Characteristics of semen stains on linen.** These stains, which we will suppose are already perfectly dried, are in general thin, faintly yellowish or greyish, little apparent to the point that to see them well, the linen must often be placed between one's eye and the light. Pressed between one's fingers, they are slightly coarse and resist as if starched, whereas parts of the linen not stained conserve their softness; they have no odor, unless moistened, whereupon they quickly emit an odor of semen. If the linen thus stained is brought near a flame, at the end of one or two minutes all the portions sullied by semen *become a tawny yellow*, whereas the other parts do not discolor unless the linen has been placed close enough to the flame to be singed: this characteristic, which did not belong to the substance of any of the morbid discharges I examined, permits the distinction on the fabric of several small whitish stains, impossible to perceive before heating. In this experiment, the semen could only have undergone a great dessication, for in leaving the linen thus yellowed in distilled water for a few hours, it loses its color and the linen acquires all the properties of solution of semen in water.

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When immersed for a few hours in cold distilled water, the stained strips moisten completely, which does not happen to the stained parts if soiled by fat. In taking care to press the strips by a glass tube from time to time, they don't delay in discoloring and unstiffening. But they become *viscous* and *emit an odor of semen*, as one can assure oneself in compressing them between one's fingers. The liquid, a milky white, troubled by a multitude of flakes and by fibrils which detach from the linen, delays a lot in clearing: if filtered and evaporated by a very soft heat in a small watch glass, phenomena occur which might be useful in the identification of semen.

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1) It is alkaline: it sometimes, however, does not reestablish the color of litmus paper reddened by acid after having been concentrated by heat. 2) If evaporated by a low flame, it presents during evaporation the viscous aspect of a gummy solution; it doesn't coagulate, although it deposits a few glutinous flakes, and its consistency is so particular it is difficult not to accord importance to this characteristic. 3) When evaporated to dryness, it leaves a semi-transparent residue, similar to dried, shining mucus, of a tawny or

\* Translation of: "Du Sperme, considéré sous le point de vue médico-légal." in *Journal de Chimie Médicale de Pharmacie et de Toxicologie* 3 (10): 469-480 (1827).

scarcely tawny color, degradable, as all nitrogenous matter at a more elevated temperature. And after being shaken for two or three minutes in cold distilled water, it divides into two parts: one glutinous, of a yellowish grey, adhering to fingers like glue, insoluble in water and soluble in potassium; the other, soluble in water. 4) The aqueous, filtered solution is uncolored, lightly yellowish or yellow, and transparent; it gives a white, flocculent precipitate with chloride, alcohol, acetate, lead subacetate and the corrosive mercuric chloride. *Pure and concentrated nitric acid* brings it a light yellowish tint if it is uncolored, but doesn't render it cloudy, whereas it precipitated or clouded the substance from the various morbid discharges designated above. Alcoholic tincture of gallnut gives rise to an abundant greyish white deposit; the aqueous solution reacted in the same way whenever used not long after being made.

Put in alcohol at 38 degrees for twenty four hours, the linen stained with semen does not unstiffen and the solution does not precipitate with water; however alcohol dissolves a small quantity of matter, for, on evaporating to dryness, a light residue is obtained.

It is easily imagined that no use can be made of observations by microscope in identifying the stains of which we speak: the spermatozoa discovered in human semen by Leeuwenhoek, frequently observed since by Gleichen, Buffon and Spallanzani, and the presence of which Prevost and Dumas have confirmed in all male animals past puberty, are no longer appreciable when, after dessication of the semen on linen, it is diluted in water for examination by microscope. Indeed, no matter what manipulation was performed in this operation, the spermatozoa are so separated in several points on their body, it is no longer possible to perceive them. It is different in distinguishing semen deposited and dried on a glass slide; the spermatozoa, not having been crumpled or separated, in this case couldn't have been more visible, although without movement; I undeniably identified them in semen dried eighteen years before. But it is especially true immediately or a little time after ejaculation, for example, a half-hour, one hour or even two hours after, that the presence of spermatozoa can be most easily confirmed; for, independent of their form, which resembles that of a tadpole, they execute very marked movements and in the extreme, one can pronounce, solely after the existence of animalculi of this form, that the solution submitted to this examination is semen, for they are not observed with the same characteristics in any other liquid. However, to leave nothing to be desired, the physical and chemical properties, of which I have already made mention, must be sought in this solution. The numerous globules, seen in the humor of the prostates of many animals, manifest no locomotor ability, are always deprived of a tail, and cannot be compared to spermatozoa.

**Matter from the discharge of chronic urethritis in several women evidently affected with syphilis.** Linen soiled by this matter presents several green, greenish yellow or yellowish stains: among the latter, a few were so little colored, that they could easily be confused with certain seminal stains;

that much more, as they emitted no odor and were coarse to the touch. Brought to a burner filled with hot coal, *these stained parts do not become yellow*. Left in cold distilled water for several hours, they discolor; the linen unstiffens and emits an odor particularly *different* from the odor of semen; the liquid was clouded with whitish flakes, and by fibrils detached from the linen. Filtered, this liquid was uncolored, transparent, and reestablished rather energetically the color of litmus paper reddened by acid. Evaporated by low heat in a small watch glass, it furnished a very abundant *albuminous coagulum*, and the solution did not offer the gummy aspect of which we have spoken concerning semen. The product of evaporation to the point of dryness was yellowish white, opaque, clumpy and degradable by fire like all other nitrogenous matter. Treated with cold distilled water and shaken for one or two minutes, it is barely dissolved: the filtered solution gives a white precipitate with chloride, alcohol, lead subacetate and corrosive mercuric chloride, and a yellowish grey one with gall nut, a little like an aqueous solution of semen; but *nitric acid, which does not cloud the latter, precipitates it in white*. The part undissolved by cold distilled water was flaky, non glutinous, and insoluble in potassium at room temperature.

**Matter from vaginal discharge in girls and women affected by acute and chronic leukorrhea.** All that has just been said concerning the discharge of venereal disease can be applied to stains which this matter forms on linen, except that they are less colored and furnish, when treated with water, a solution in which the reagents already noted provoke much less apparent precipitates.

**Matter from a discharge of the urethral canal, in a case of an internal one-eyed fistula, the sequel of several external fistulae.** The linen is stained in greenish yellow; the matter was deposited forty days before. It is starched, coarse to the touch, and has no odor in the stained parts; it *does not* yellow like semen when heated. Put in water, it discolors, unstiffens, acquires a particular odor *very different* from the odor of semen. At the end of a few hours, the liquid, slightly troubled, is filtered, to be evaporated at a low heat. Before its reduction to dryness, it reestablishes the color of (litmus) paper reddened by acid; it does not coagulate, and *in no way presents the viscous aspect* of gummy solutions when heated. In treating the very light-yellowish residue coming from evaporation to the point of dryness with cold distilled water, a part is dissolved; the filtered solution gives a white precipitate with chloride, alcohol, lead subacetate, the corrosive mercuric chloride and *nitric acid*, and a yellow one with gall nut.

**Matter from a discharge of the urethra in venereal disease, five days after cauterization.** The stains this matter forms on linen quite resemble those of semen; the sullied parts were coarse to touch, starchy, without odor; but *they didn't yellow on heating*. Cold distilled water, had discolored and softened the stained portions after a few hours; it had developed an odor different from that of semen. The liquid, clouded by flakes and by fibrils gave a yellowish, alkaline residue, simi-

lar to dried egg white, upon being evaporated to dryness. The residue did not appreciably dissolve following two minutes agitation in cold distilled water; in addition, the filtered solution remained transparent when added to chlorine, nitric acid, mercuric chloride, alcohol and gall nut. Now, it is well known that aqueous solutions of semen give precipitates with all these reagents, except for nitric acid.

**Whitish lochial material, called "laiteuses."** This material forms stains of a dirty yellowish-grey on the linen, having some resemblance to seminal stains; nevertheless, *they do not yellow* upon heating. Treated with cold distilled water for a few hours, they detach and the linen discolors and softens; the scarcely clouded liquid, filtered and evaporated, does not coagulate or deposit flakes, and presents rather the aspect of a gummy solution, a bit like semen treated with water and heated. It is alkaline and recolors litmus paper reddened by acid: however, it becomes colored, and yellows *proportionately to the concentration of the solution* and the dried product is a *deep yellow* similar to "colle à bouche fondue," which doesn't happen with the dissolution of semen. In shaking the dried product for two minutes in cold distilled water, it dissolves in part; the undissolved portion is flocculent, of a deep yellow, and soluble in potassium; the dissolved portion, after filtration, is yellowish and gives an *abundant* precipitate with *nitric acid* and gall nut; chloride, alcohol and lead subacetate give a precipitate and render the solution opaline.<sup>1</sup>

**Characteristics of fat stains.** They present a fatty aspect, are neither coarse to touch nor starched, and when heated they expand without yellowing. Moreover, they emit their well known odor. Placed in cold water, the linen soiled by the *fat doesn't moisten* in the stained parts; *the fat is in no way dissolved*. If left for a few hours in cold alcohol measuring 38 degrees Baumé, the fat is removed, the alcohol holding it in solution. Water gives a white precipitate and when evaporated to the point of dryness it furnishes a fatty residue. Finally, if the linen concerned is immersed for a while in a solution of potassium, soaplike droplets are seen on the surface of the solution, and the solution furnishes a fatty, white precipitate if a few drops of acetic acid are added.

**Linen stained with nasal mucus.** The stains are deep yellow, though the mucus was white when deposited on the linen. Left in cold distilled water for a few hours, it discolors; the fabric is cleaned, the liquid becomes troubled, whitish and flocculent. It is filtered and evaporated by low heat.

<sup>1</sup> In evaporating to the point of dryness the various aqueous solutions furnished by the matter of discharges, of which I have spoken up to now, it is easy to see that the greater part of them furnish an abundant albuminous coagulum, such that the dried product is formed almost entirely by albumin. Then, as this product dissolves in notable quantity in cold distilled water, since chloride, alcohol, gall nut, etc. give a precipitate, I wanted to find out to what point albumin coagulated by heat could dissolve in water. In a watch glass, I evaporated to the point of dryness egg white, diluted in water and filtered; after coagulation, the solution furnished a solid product, which had been dried and shaken for two minutes in cold distilled water. It was once again filtered and the solution gave a precipitate with alcohol, chloride and gall nut; nitric acid also clouded it.

When fairly concentrated, it recolors litmus paper reddened by acid. It presented no trace of *coagulum* during evaporation, and furnished a very small quantity of a whitish, transparent, granulous-like matter. Shaken for one or two minutes in cold water, this matter scarcely dissolves and leaves numerous whitish flakes. The filtered solution is limpid and gives a rather abundant precipitate with chloride, *nitric acid* or alcohol; aqueous solution of gall nut or lead acetate do not cloud it.

**Linen stained with saliva.** Several linens stained with saliva, coming from six adult individuals, were examined with care: the stains were the result of the reiterated application of saliva on the linen. The characteristics presented not always being the same, we feel it necessary to describe the details observed.

A. Some of the dried stains were starched, coarse to touch and *yellowish*, although the saliva was white coming from the mouth; during dessication, it manifested a particular, disagreeable odor. In exposing the stained parts to heat, those, for example, hardly presenting a yellow tint, acquired a more intense color, and resemble seminal stains treated in the same way. Left in cold distilled water for a few hours, they unstiffened and the linen emitted an odor of semen, especially when pressed between one's fingers. After having been filtered and subjected to a low heat, the very alkaline liquid, troubled by a multitude of flakes, did not coagulate, and furnished a rather abundant yellow residue. This separates into two parts after shaking in cold distilled water for a minute or two: one part insoluble, in the form of thin, yellowish pellicles similar to mucus; the other soluble, which becomes opaline with chloride, nitric acid, or alcohol and with which lead subacetate gives an abundant precipitate, whereas aqueous solution of gall nut doesn't cloud it.

B. Here the linen was white, starched and almost without odor; heated, it *didn't yellow*. Treated with distilled water as was the preceding, it presented a light odor which was *nothing like semen*; the liquid was troubled, flocculent and alkaline. Heated after filtration, it didn't coagulate, and evaporated in the manner of gummy solutions; the product of evaporation was yellowish, semi-transparent and salt-like. Shaken in distilled water for two minutes it separates from the mucus flakes, or rather, the pellicles. The filtered solution *did not become opaline again* with chloride, nitric acid, alcohol or aqueous solution of gall nut.

C. This type resembled the preceding except that heat *yellowed* the linen and the solution became troubled by evaporation, as if it had been albuminous.

It is evident from the preceding: 1) that it is hardly possible to confuse semen stains on linen with those of fat, nasal mucus or the matter of various discharges coming from the vagina or urethral canal; 2) that it is a matter of confirming only the *whole* of the characteristics we have presented in speaking of semen; 3) that it is *sometimes* not so easy to differentiate a seminal stain from a stain formed by saliva; but that it is, however, possible to succeed, the latter liquid not presenting, under any circumstances, *all* the character-

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istics of semen. Besides, it is hardly probable that shirts, which one is most often called upon to investigate, have been stained with saliva, especially since deposits of several repetitions are necessary to form an appreciable stain with this liquid, and since it is necessary to wait until the first parts applied have dried, which requires a lot of time.

## Microscopical Examination of Dried Semen on Linen or on Material of Varied Nature and Color\*

Dr. H. Bayard

Newton was asked how he had made all his discoveries; he replied: *In always searching, and in searching with patience. I have followed this counsel. . . . Si parva licet componere magnis.*

### Preface

/134 When addressing this memoir to the society of the *Annales d'hygiène et de médecine légale*, I deposited it before January 1, 1839 in conforming to the conditions of the gathering; but I pursued my research, however, in order to modify my analytical procedures to confirm the presence of spermatozoa without breaking their tails. By means of filtration, I obtained the results I was seeking. Last March, I was called before the society of the *Annales* to repeat a few microscopical experiments, and I verbally communicated the new method of microscopical examination, whose details I present in this memoir.

/135 For a long time, the inadequacy of chemical analysis to determine with certainty the nature of semen stains has been recognized. Now, microscopical analysis can furnish certain results which chemistry doesn't offer in legal assessments dealing with crimes of rape, indecent assault and in certain cases of violent death.

Paris, May 15, 1839

### Microscopical examination of dried sperm on linen or on material of varied nature and color

Use of the microscope in medico-legal assessment was first recommended by Orfila<sup>1</sup>, to determine the nature of sperm in cases of rape and indecent assault; his research did not furnish him with satisfactory results, for he says: *no use can be made of microscopical observation in the identification of seminal stains.*

Since that time, this investigative method appears to have been neglected in its applications to legal medicine, and it is only lately that many forensic physicians have noted the importance and utility of microscopical observations.

Ollivier (of Angers) is the first to have made a conclusive application of the microscope in a medico-legal assessment.

/136 In June, 1837, he was charged with determining *if there didn't exist hair adhering to the iron of an axe seized at the home of an individual indicted for murder and, in the case of affirmation, to indicate the color of these hairs.*

\* Translation of: "Emploi du Microscope en Médecine Légale. Examen Microscopique du Sperme Desséché sur le Linge, sur les Tissus de Nature et de Coloration Diverses".

in *Annales d'Hygiène Publique et de Médecine Légale* 22: 134-170 (1839).

He recognized by microscope that the *filaments* submitted for examination were *fur*, differing completely from *hair*; whereas they perfectly resembled the fur of horse, beef or cow comparatively examined; a judicial inquiry confirmed the correctness of his observation.

Ollivier (of Angers) reports in a note added to the article I have just cited that in June, 1838, in a legal assessment, with which he was charged along with Labarraque and Gaultier de Claubry, having as the object of examination a large amount of denatured, adulterated opium, Gaultier de Claubry confirmed by microscopical examination not only the adulteration, but he also discovered by this means the different method of extraction of opium in Smyrna and of opium in Egypt.<sup>2</sup>

In a meeting of the Academy of Medicine on November 20, 1838, A. Divergie read a note on the characteristics of hanging a living man, and added two new characteristics: the first consisting of the presence of spermatozoa in the urethral canal; the second, the state of congestion of the genitalia.

It is this small number of facts to which use of the microscope is limited in legal medicine up to the present.

Now that one is not content with studying in visible texture of organized bodies, but more with discovering un- 137/ awares, so to speak, their mode of primitive formation, and knowing their intimate composition, the microscope, due to the modern perfections in its construction, will serve to extend the limits of science.

Doctor Donné, in two memoirs appearing in 1837, presented important microscopical research *on the nature of mucus and the substance of discharges from male and female genital organs and on spermatozoa.*

In his last work, Donné was particularly interested in the fluids of "economy", which are appropriate for maintaining the life of spermatozoa for a more or less long time, and from considerations of some of them, he deduced causes of sterility in women. This research is not especially applicable to legal medicine, but I must hasten to point out it is fruitful in application, and will yield priceless information.

Among the ancient and modern authors occupied with the study of human spermatozoa, none, Orfila excepted, observed them with the same objective as myself.<sup>3</sup>

/138 Gleichen, Spallanzani, Lewenhoeck, Peltier, Prévost and Dumas, Donné, etc., have observed living spermatozoa, and most of these authors were studying them in a physiological context, seeking to determine their influence on generation.

I considered spermatozoa from an entirely different point of view; I observed them *dead* when they were dessicated as was the liquid in which they were suspended.

The importance of this type of research is now understood, in cases of rape or indecent assault, where stained material or fabric is submitted to examination by experts, to determine the nature of the observed stains.

Up to today, reliance has been placed only on results of chemical analysis; these analytical methods, recommended with wisdom by science, are, however, coarse and little conclusive.

It is true that some microscopical experiments have been attempted, eleven years ago, but with no success, due to the imperfection of instruments and procedures. I devoted myself to new experiments and the CERTAIN results I obtained by microscopical analysis permit me to communicate them. In addition, I am assured that a certain number of obscure questions in legal medicine can be cleared up by this mode of investigation.

It will be very important to determine if spermatozoa exist in all ages in men; I intend to study this question which is of interest at the same time to physiology and to legal medicine.<sup>4</sup>

### First Section

/139 This memoir is composed of three sections: in the first, after explaining the facts which led me to look into new procedures, I successively study the action exerted in cold and heat on dry semen by:

- distilled water,
- common water,
- saliva,
- urine,
- blood,
- milk,
- alcohol,
- solutions of soda,
- sodium subcarbonate,
- sodium subphosphate,
- potassium,
- potassium subcarbonate,
- ammonia.

I end with the enumeration of characteristics presented by dried semen on linen.

### Second Section

The second section comprises three series of experiments; but before detailing them, I recommend various procedures I successively employed before resorting to filtration; finally, I set forth this mode of analysis which appears to me the most complete and most certain.

### First Series of Experiments

- A. Examination of linen stained by simple dried vaginal mucus.
- B. Examination of linen stained by semen.
- C. Examination of linen stained by vaginal mucus after the act of coitus.
- D. Examination of linen stained by vaginal mucus, collected eight hours after coitus.

### Second Series of Experiments

- E. Examination of linen stained by simple vaginal mucus.
- F. Vaginal mucus collected between glass slides.
- G. Examination of linen stained by semen.
- G<sup>1</sup>. Semen collected between glass slides.
- H. Examination of linen stained by vaginal mucus after coitus.
- H<sup>1</sup>. Vaginal mucus, after coitus, collected between glass slides.
- I. Examination of linen stained by vaginal mucus nine hours after coitus.
- I<sup>1</sup>. Vaginal mucus, collected between glass slides, nine hours after coitus.

### Third Series of Experiments

- J. Examination of linen stained by semen two months before.
- K. Examination of linen stained by semen one, two and three years before.

### Third Section

The third section is composed of a large number of microscopical experiments on stains of semen, of vaginal mucus with semen, dried on material of

- cloth,
- cotton,
- wool,
- silk,

which vary in color.

My research at this moment concerns whether the characteristics Doctor Donné assigned to mucus and to the substance of various discharges of male and female genito-urinary organs can be recognized on linen and fabric.

The experiments I have already done on this subject permit me to expect success and confirm in part the important discoveries of this able observer.

Paris, December 25, 1838

### First Section

During a legal investigation against Sir Bengnet, indicted for the murder of his mistress, he claimed that the night or morning preceding the murder, the girl, Lecluse, had had sexual intercourse with a stranger, and the despair at being thus wronged by the woman he was to marry in a few days brought him to kill her.

I was charged, with Ollivier (of Angers), to submit any

liquid in the genital parts of this girl to special examination in order to see if there wasn't any trace of semen.

To proceed with this investigation, we had carefully removed the uterus and vagina from the cadaver, in such a way as not to perturb the walls of this canal; it was incised lengthwise with caution, and we wiped the whole inside surface, those parts touching the cervix included, with a very white linen cloth. The linen, moistened by these mucus substances, which were rather abundant, was dried, in order to be submitted to various methods of investigation later.

142 To facilitate this examination, I devoted myself to several series of experiments with the objective of seeing if the presence of spermatozoa can be confirmed by microscopical examination on linen stained by human semen or vaginal liquid mixed with semen and dried. In an article entitled *Sperm considered from the medico-legal viewpoint*<sup>5</sup>, Orfila noted chemical and physical characteristics by means of which the presence of seminal stains on linen, or stains from substances of various discharges, can be confirmed.

This author expresses himself thusly, page 473. ". . . It is easy to realize that no use can be made from microscopical observations in the recognition of seminal stains: spermatozoa discovered by *Lewenhoek*, frequently observed since by *Gleichen*, *Buffon* and *Spallanzani* and whose presence *Prévost* and *Dumas* confirmed in all male animals past the stage of puberty, are no more appreciable when, after having dried the semen on linen, it is diluted in water for microscopical examination. Indeed, no matter what manipulation is used in this operation, the spermatozoa are so separated in many parts of their body, it is no longer possible to perceive them. It is different in distinguishing semen deposited and dried on a glass slide; the animalculi, being neither crumpled nor separated, in this case couldn't be more visible. Although without movement, I recognized them perfectly in semen dried for eighteen years. But it is especially immediately, or a little time, after ejaculation, for example, a half hour, one hour and even two hours afterwards, that the presence of the animalculi is easy to confirm. For other than their form, resembling a tadpole, they execute very marked movements, and, in the extreme, the solution submitted to examination can be considered semen from the soil existence of animalculi thus formed, for they are not observed with the same characteristics in any other liquid. . . ."

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The opinion expressed by Orfila in 1827 did not stop my research and, profiting from perfections in microscope construction since that time, I obtained, as can be seen in this memoir, more successful results.

The procedure recommended by Orfila is the same as I have seen still very recently employed, and it is easily understood that it cannot confirm the presence of spermatozoa. If the stained linen is placed in water, in fraying and separating the material, the spermatozoa are broken and the debris is hardly discernible, no matter the magnification of the microscope.

Examination of sperm collected between glass slides right

after ejaculation and of sperm gathered in a sizable quantity in a capsule to preserve it as liquid for about six hours, brought me to the use of procedures I will detail below.

I observed that zoosperms between glass slides conserve their life and movements as long as the mucus in which they are swimming stays fluid, and as it became cold and dry, they lost their mobility and exerted only vibratory oscillations, which stopped right after complete agglutination of the mucus, which took place at the end of two or three hours.

I've no need to remark that spermatozoa are always visible 144. between glass slides, for at the moment they are interposed, the mucus is dispersed in an excessively thin layer, the agglutination of which is not harmful to observation. In a capsule where the sperm solution was abundant enough to conserve its fluidity for about ten hours, I could confirm the life and movements of spermatozoa up to the last instant.

Starting with these observations, I devoted myself particularly to recognizing the action of several preservation liquids and a certain number of chemical agents on dried sperm, to distinguish those which disengage the zoosperms most promptly and completely from the muco-glutinous matter without altering them from those which, on the contrary, alter the form of, or destroy, the spermatozoa.

For these attempts, I used semen in which I had identified movements of the animalculi for ten hours; the semen had been exposed to the atmosphere and had dried in the capsule.

In the central part of the capsule the semen is a yellowish color whereas in the other parts, the tint is greyish; it is very dry and detaches in the form of powder.

I was interested in submitting this seminal powder to microscopical examination, using a magnification of about three hundred fifty. A few animalculi, very recognizable by their form, were free and entirely disengaged from the mucus matter; but the greater part were surrounded by a rather substantial thickness, such that the bodies were semi-opaque, and the content was distinguished only with great difficulty.

§I. Action of distilled water. A drop of distilled water is 145 placed on this seminal powder; after a few minutes of maceration, the semen swells, disseminates in the liquid, and under the microscope, a large number of free zoosperms in the middle of irregular, transparent bodies are seen. Lightly heated, these bodies dissolve a bit, and permit perception of the imprisoned zoosperm.

I couldn't better compare the fragments of glutinous mucus than to icicles formed by the cold enveloping all the substances suspended in the water. As previously, they are dissolved by heat and release the foreign bodies imprisoned there.

This dissolution is not, however, complete enough such that there are no remaining fragments of mucus; but they are transparent and it is in their midst that the animalculi are perceived; prostatic monads can also be identified, having a globulous form without a tail. Their volume is infinitely more considerable than that of zoosperm from which they are easily distinguished.



**§II. Action of common water.** Common water, hot and cold, acts like distilled water. Experiments I've done on river and well water permit me to confirm some rather appreciable differences when the qualities of the water vary; for example, the alkalinity of water activates dissolution of mucus.

A general remark, one which promotes the preference for distilled water, is that common water holds a great number of substances in suspension which deposit between the slides and hinder microscopical examination.

**§III. Action of saliva.** As soon as dried semen comes in contact with saliva, it swells and disseminates more rapidly than in distilled water. Under the microscope, the mucus is divided into transparent fragments, which partially dissolve if lightly heated; the zoosperm are apparent, but there are very few of them free and they are surrounded by mucus.

I haven't noticed that saliva exerts a singular action on *dead* zoosperm, an action noted by Doctor Donné on *living* animalculi; *their body doesn't become contorted, so that the tail forms a type of knot or eyelet.* In all my experiments, the tail maintained the direction it had at the moment of contact with the saliva.

**§IV. Action of urine.** Sperm disseminates more rapidly in urine than in saliva, the mucus fragments divide more and are more transparent; prostatic monads are free and visible in great number, heat increases the dissolving action a bit, the spermatozoa are very visible and almost totally disengaged from the mucoglutinous matter. If the glass slides are left to cool, at the end of a few minutes crystallizations of different urinary salts form, which does not hinder the identification of spermatozoa.

I repeated a great number of times these experiments of the action of saliva and urine on semen, because I was astonished that urine, which is ordinarily acid, more easily dissolved the mucus, or, to put it more precisely, rendered the spermatozoa visible more rapidly than saliva, which is an alkaline liquid. I always noted the same results, however, even though I used the urine of many people of differing age and sex. The explanation of this difference can be found, it seems to me, in the presence of the abundant mucus existing in saliva and which is added, as it were, to the glutinous mucus of sperm, whereas there is not an appreciable quantity in urine.

**§V. Action of blood.** It is known that blood, far from exerting a deleterious action on the zoosperm, appears to preserve their life; I have no other purpose in this research than to confirm if the presence of blood hinders microscopical examination. I noted that zoosperm were perfectly distinct in the midst of blood cells; it suffices to add a drop of distilled water and to shake the slides a bit, so that in the movements, whole zoosperm can be identified.

I will return later to the importance of examination by microscope in determining the nature of stains presumed mixed with blood.

**§VI. Action of milk.** I used mother's milk, and I observed that dried semen put in contact with milk swelled very little

and didn't disseminate, which is explained quite well by the multiplicity of milk globules; but as soon as a drop of distilled water is added, the glutinous mucus of semen quite promptly divides, prostatic monads appear, then the zoosperms differentiate themselves by their elongated tail.

**§VII. Action of alcohol.** *Pure* alcohol contracts the glutinous mucus of semen, and no trace of zoosperm is seen; if alcohol is added to a solution of semen in distilled water, this phenomenon does not occur. And as soon as it is gently heated, the mucus fragments divide, becoming transparent, and zoosperm disengage themselves. I made numerous attempts at determining the action of alcohol and I confirmed that *one drop of alcohol to ten drops of water* is the proportion which most activated the division and transparency of muco-glutinous fragments. This dissolving action of alcohol should not be astonishing; it had been noted by Orfila who said in his memoir (page 472) . . . "Placed in alcohol at 38 degrees for twenty-four hours, the semen-stained linen doesn't unsoften, and the solution doesn't precipitate with water; however, alcohol dissolves a small amount of matter, for in evaporating to the point of dryness a light residue is obtained."

Orfila's observations are noted when linen stained with semen is left to itself after being saturated with alcohol. But if lightly heated after the addition of distilled water, the stained linen *loses its stiffness* and recaptures it to a lesser extent after the complete evaporation of distilled water. If the liquid of the solution is submitted to examination by microscope, and particularly that gathered in the bottom-most part of the capsule, *spermatic animalculi are found.* It is understood that chemical procedures alone cannot contradict such results.

It is with alcohol that I began a multitude of experiments which, by their successful results, confirmed the certainty of the procedure, and since I've been able to compare the action of several other reagents to this chemical agent, I don't consider it to be of any less genuine value, for the proportions are easily measured and its *useful action* endures for a much longer time. I will have occasion to return to this subject in the third part of this memoir, when I present my research on semen stains of cloth of various nature and color.

**§VIII. Action of sodium and a few of its salts.** Reflecting that sodium exists as a salt, dissolved in the bodily humors, and that their alkaline state is undoubtedly due to its presence, I repeated many experiments with this substance, either *pure* or as subcarbonate and subphosphate.

In the *pure state*, sodium solution provokes contraction, shriveling of glutinous mucus, and zoosperms are not perceptible; but, remarkably, prostatic monads are free and appear more voluminous than in solutions of distilled water or urine.

If as sodium, sodium subphosphate, or sodium subcarbonate is added to a lightly heated solution of sperm in distilled water, the mucus rapidly dissolves, spermatozoa and prostatic monads appear; but if the appropriate proportion of reagents hasn't been used, the spermatozoa are no longer found at the end of a few hours, while the prostatic

monads are visible.

150 After much trial and error, the proportion which appeared to produce the best effect is 1:20 of the concentrated solution, i.e. one drop of sodium (subcarbonate) solution for twenty drops of distilled water.

Despite the difficulties encountered in use of this reagent, I don't think it should be rejected, for its action is rapid and very advantageous if the proportion is followed closely.

§IX. **Action of potassium.** I used a solution of potassium subcarbonate in the same proportion as sodium and obtained the same effect; I will limit myself to mentioning this, without giving all the details which will only recall what I have previously said.

§X. **Action of ammonia.** *Pure* ammonia has the same action on semen as *pure* alcohol or pure sodium; but if added to a solution of distilled water and gently heated, the results obtained are conclusive.

On contact with ammonia, the mucus rapidly dissolves; the zoosperm are not altered and are discernible for a rather long time; but at the end of twenty-four to thirty-six hours, on examination of the slides between which the dissolution was performed, zoosperm are no longer found. In evaporating, the ammonia promptly dried the slide, or else this alkali destroyed the animalculi. In any case, they are no longer seen.

151 The proportion in which this reagent can be used required many attempts. I used one sixteenth of the concentrated solution, *one* drop of ammonia to *sixteen* drops of solution; and, I repeat, even in conserving this proportion, I found no trace of zoosperm after forty-eight hours.

Due to its rapidity, the action of ammonia must be preferred to the reagents already studied when the research to be performed must be done in a few hours. This chemical agent completely dissolved blood. Its use should not be forgotten when a semen solution submitted for examination is to be separated from blood.

In summing up all the preceding observations, it is seen: 1) that distilled water or common water dissolves a part of the seminal substance and that, in gently heating the macerated material, the division of mucus fragments and their transparency is increased and zoosperm are thus rendered more visible; 2) that spermatic animalculi become visible in saliva and in urine, and that these liquids do not alter them, likewise for blood and for milk; 3) that *concentrated* alcohol, sodium, potassium, ammonia, far from dissolving mucus and disengaging zoosperm, cause a very marked contraction and destroy them; that these reagents, employed in *appropriate quantity* and added to the macerated seminal material have a very remarkable dissolving action, by which the animalculi are rendered apparent.

To avoid confusion in the presentation of my research, I previously spoke only of the action of various liquids on *dried semen*; but the objective I set for myself is to confirm that *identification of dried seminal stains on linen can benefit from observation by microscope.*

If linen stained by semen and dried is examined, charac-

teristics noted by all researchers can be easily recognized; 152/ they are the following:

The stains are thin, of a greyish or yellow-red-brown color, sometimes not very apparent, and, in certain circumstances, of a shiny, gummy appearance. The stains are stiff to touch, the linen rigid as if starched. A remark very important to make is that these characteristics are most usually observed on the surface which had been moistened by the semen and, if the linen is thick, the surface opposite to the stain presents no change in color.

When the strips (also stained) are macerated for a few hours in cold distilled water, they are completely moistened, which does not take place in fat stains; the linen loses its color and unstiffens. The liquid becomes slightly troubled, if the semen is in appreciable quantity; fibrils are detached from the linen and deposit with small flakes at the bottom of the capsule. A spermatic odor is exuded if longer strips are used; if not, it is difficult to appreciate.

During this maceration, *it is necessary to take care not to press the stained linen with a glass tube or any other body nor to dilute it in water*, for what had been noted by Orfila will inevitably happen; *the spermatozoa will be so separated in many points on their bodies, that they will not be identifiable.* If, on the contrary, precaution was taken not to crumple the linen, it suffices to aspirate a few drops of the maceration mixture with a pipette, choosing preferentially the lowermost part of the capsule, and to interpose the liquid between two glass slides for microscopical examination. A few free zoosperm will be identified and a greater number are imprisoned in fragments of glutinous mucus. At this point, using a low heat and one of the reagents, such as alcohol, 153 sodium phosphate, potassium, or ammonia, causes a much more complete dissolution of the mucus to be brought about and a greater number of zoosperm liberated.

These zoosperm can always be recognized by their particular form, just about that of a tadpole. The numerous globules perceived in the liquid of the solution are prostatic monads which are *always* deprived of a tail and are of a much more considerable volume.

## Second Section

Before presenting the experiments which are the object of this section, I think it would be useful, to avoid continual repetition, to detail the procedures I found the most advantageous in my research with the microscope. In the first part of this memoir, I presented some considerations which touched on a few of these details; I will, therefore, be as concise as possible.

**First procedure for recognizing the presence of spermatic animalculi on linen or fabric stained by semen and dried.** It is necessary to place the strips of stained linen or fabric in a glass capsule,<sup>6</sup> taking care, as I've already recommended, not to press or crumple, and still less, to separate the fabric. 154 They must be moistened with water and left to macerate during several hours, then *gently heated* on the flame of a spirit lamp, taking care not to bring the liquid to a boil.

**Second procedure.** The analytical method previously presented seemed defective to me in many respects; and forced as I was, by requirements of the meeting, to deposit my manuscript before January 1, 1839, I had to limit myself to presenting the first procedure. I did not stop, however, doing new research and I will stop finally at the method of examination that follows, which I presented to the Society of the Annales d'hygiene et de médecine légale, when I was called last March to repeat before it some of the experiments cited in my memoir.

In doing *chemical analysis* of linen stained by semen, I remarked that the maceration liquid became, by filtration limp and transparent, as cloudy and opaline as it was beforehand and that this change was due, as can be easily imagined, to deposit on the filter of all the animal and foreign matter undissolved in water. I at once applied this observation of microscopical research, and I examined the matter thus deposited on the filter. I distinguished a multitude of spermatic animalculi entire and complete for the most part, but enveloped in mucus or foreign bodies. With the help of heat and some of the reagents already cited, I could disengage the zoosperm which I had thus obtained complete and isolated.

It is known that spermatozoa, due to their specific gravity, gather together at the bottom of vessels containing the liquid holding them in suspension; it is natural then that they deposit on the filter. I ascertained that the spermatozoa were stopped by a simple sheet of filter paper, a fact already recognized, I believe, by Prévost and Dumas.

**Mode of analysis** — 1) Detach with scissors and carefully remove a portion of the presumed seminal stains; do not crumple the fabric, and place it in a test tube.

2) Bathe the stained fabric in distilled water, and let it macerate for twenty-four hours.

3) At the end of this time, filter this first liquid. Place the stained fabric, already macerated, in a porcelain capsule, moisten with distilled water, and heat by the flame of a spirit lamp until the liquid acquires a temperature of +60 to +70 degrees centigrade. Filter this liquid. Finally, treat the stained fabric with alcoholic water or ammonia in water and filter the diluted solution.

4) When the filtration is finished, cut the filter paper a distance of one thumb from its edge and turn it over on a watch glass, or preferably on a flat glass dish; moisten the filter thus turned over with dilute alcohol or dilute ammonia, which dissolves the mucus and entirely detaches the deposit. If some fatty matter is found mixed in, a couple of drops of dilute ether is used.

Examination by microscope of the capsule or flat glass dish identifies *whole* spermatic animalculi, *without breakage* of the tail, and isolated from the mucus.

I have already performed numerous applications of this method of examination, particularly in *eleven* legal assessments with which I had been charged since February, conjointly with Drs. Olliviers (of Angers), Moreau and Chevalier. Microscopical examination *gave certain results* each

time, which chemical analysis, comparatively performed, did not always give.

The solution obtained is divided into several parts and to each is added 1:10 alcohol, 1:20 sodium or potassium, 1:16 ammonia; after a few minutes a deposit forms on the bottom of each capsule. A few drops must be aspirated with a pipette, and placed between two slides which are placed on the stage of the microscope, and a magnification of 350–600X is used.

Stains of a fatty type are observed between the two slides; these are the stains which must be carefully observed, and here are found zoosperm, which does not hinder, however, the seeing of a multitude of suspended corpuscles in the liquid, and even perhaps some free zoosperm at other points on the slides. A few drops of the liquid thus prepared can be placed on a slide and left to evaporate; if the deposit thus formed is submitted to microscopical examination after complete dessication, the zoosperm are easily identified. In thus working with only one slide, the objects at which one is looking are lighted much more vividly, which is very advantageous when a lit room is being used for sketching.

#### First Series of Experiments

It was not sufficient for me to confirm the presence of spermatic animalculi in dried seminal stains on linen; I wanted to examine stains dried on linen and mixed with vaginal mucus which flowed during and after the act of coitus.

I succeeded in acquiring such linen collected with care, and devoted myself to the research which is the objective of this second part.

**A. Examination of linen stained by simple dried vaginal mucus.** These linens were used to wipe the genitals of a healthy woman, who had no discharge, and who had not experienced coitus for over fifteen days.

Rose and light yellowish stains are observed on the linen, more colored on one of the surfaces than on the opposite; the fabric was not starched, but it felt a bit stiff to the touch and seemed swollen. The strips are macerated in distilled water; blue litmus paper is dipped into the maceration mixture, and it reddens a bit, but very weakly; the acidity, however, can be confirmed.

Examined by microscope between two slides, this liquid appears to be composed of a large number of irregular bodies, of which I could not exactly identify the oval form described by Donné (p. 17, *Recherches sur la nature du mucus*), but I determined without doubt that they looked like small scales. In addition, I observed a good number of rose-colored corpuscles, which did not show a regular form. There was nothing resembling animalculi, of which I made certain in submitting this liquid to the action of the various chemical agents already cited, which dissolved the mucus, altering the form of the scales, but there appeared no bodies analogous to sperm or prostatic monads.

**B. Examination of linen stained by semen.** These linens wiped the genitals and penis of a man right after coitus.

Greyish, starched, limited stains were noted; these stains,

cut out and placed in a capsule, were treated according to the recommended procedure and submitted to the action of varied reagents. Examination by microscope identified a large number of zoosperm and a multitude of prostatic monads.

**C. Examination of linen stained by vaginal mucus after coitus.** These linens were saturated by vaginal mucus a little after the act of coitus; in these experiments as in all those reported in this memoir, these linens were *dry* when examined.

The linen presented a light yellowish tint at the stained points; it is firm, starched, presenting the characteristics of semen-stained linen.

The solutions suspend the zoosperm and the prostatic monads; but the papulae and the scales identified in simple vaginal mucus are observed here and are, for the most part, adherent to the spermatic glutinous mucus.

**D. Examination of linen stained by vaginal mucus collected eight hours after coitus.** It was of interest to me to determine how many hours after coitus spermatic animalculi can still be found in vaginal mucus; I obtained mucus collected from a woman eight hours after coitus without any bathing of the genitals.

159 The linen was stained greenish yellow and was firm, but not rough to touch.

On examination by microscope, I observed a large number of colored corpuscles suspended in vaginal mucus characterized by the scales, and there I found entire zoosperm and prostatic monads more or less ensnared by the plastic matter.

### Second Series of Experiments

To verify experiments done in the preceding series, I obtained the same liquids which stained the linen, but collected samples at the same time, between glass slides. It is known that spermatozoa interposed between glass slides can be preserved for a number of years; the examination of what was enclosed between the slides furnished me with points of comparison, and I confirmed the accuracy of my first experiments.

I will not report here the details of these experiments, for that would be a repeat of what I have already presented at length. I successively and comparatively examined:

- F. Linen stained by simple vaginal mucus.
- F<sup>1</sup>. Vaginal mucus collected between glass slides.
- G. Linen stained by semen.
- G<sup>1</sup>. Semen collected between glass slides.
- H. Vaginal mucus collected on linen after the act of coitus.
- H<sup>1</sup>. This mucus between slides.
- I. Linen stained by vaginal mucus, nine hours after coitus.
- I<sup>1</sup>. This same mucus between slides.

160 In all these experiments, I identified spermatic animalculi in the liquid of the solution, and at the same time I saw them preserved between the slides.

I wanted to be sure as to the number of hours spermatic

animalculi adhere to vaginal walls, even when washing has been done with simple water. I have identified some in vaginal liquid *sixty-two hours* after coitus; but they were no longer perceptible *four hours* afterward if the woman bathed with aromatic water of eau de cologne. It is probable in the last case that the glutinous matter surrounding the zoosperm and holding them fixed to the vaginal wall at its entrance, was dissolved by the alcohol, and that these animalculi were washed out by the liquid used to carry out the bathing.

### Third Series of Experiments

In all the preceding, I worked on linen stained a few days before. I owe to the kindness of A. Chevallier, member of the Academy of Medicine, the opportunity to experiment on linen stained a much longer time before. This chemist procured for me linen stained by semen *two months, one year* and nearly *three years* before.

**J. Examination of linen stained by semen two months before.** This linen is a fabric of very fine, very white flax; the stains are greyish, starched, the fabric folded, the folds very stiff to touch.

After maceration of a strip of this linen in distilled water and its submission to various methods of analysis, a large number of prostatic monads and zoosperm are perceptible; a few of the animalculi were broken, but a few can be seen 161 which are not entirely dissociated.

**K. Examination of linen stained by semen a year and two years before.** I did experiments on *five* of these linens, two were of flax, the three others were of cotton, all of them very starched, deeply colored in yellow, one of them rough to the touch and giving the sensation of granulations.

The liquid of maceration has a lightly opaline tint. The whitish flakes, held in suspension for a little while, as well as a type of fine, granulated powder, deposited at the bottom of the capsule.

Under the microscope, the colored corpuscles of irregular form, the glutinous matter of little transparency and prostatic monads are perceptible.

The use of alcohol, of sodium phosphate, . . . accelerate the dissolution, and a rather large number of whole and broken spermatozoa are perceptible, and some whose tails are circularly distorted; the prostatic monads are very apparent.

The glass slide moistened with the solution is dessicated by exposure to the atmosphere, and I am quite surprised to identify under the microscope sodium phosphate and ammonia crystals in pyramids of four faces and truncated peak; I repeated the experiment by leaving a simple maceration liquid of one of these linens open to the atmosphere, and the crystals are reproduced; I am convinced that this salt exists in a state of solution at the time of semen ejaculation.

### Third Section

It is not only on linen but on fabric very different by their 16 nature and color where semen stains will have to be investi-

gated; it thus appeared important to me to study them when they are dried on material of *cloth, cotton, wool, silk*.

I have previously pointed out the physical characteristics of seminal stains dried on material of cloth and of cotton, either unbleached or white; I will not return to the details already reported, but I believe it useful to present a few of my remarks which I made on material tinted of different colors.

**Examination of blue twill duck stained with semen.** This material of blue color is shiny, glossy, and supple, although firm almost in its entirety.

There are some parts *dulled* by a dried, whitish coat; at these points the material is starched and does not show the suppleness observed in neighboring points.

Maceration removed the dull color of the stained points on the duck; some fibrils as well as other corpuscles deposit at the bottom of the capsule. The liquid has a bluish tint; treated with alcohol, it doesn't change color and spermatic animalculi can be identified.

If ammonia is used, this reagent alters the strands of threads, without, however, hindering microscopical investigation.

Strands of thread, or their fibrils, are easily differentiated from spermatic animalculi, for the volume of the latter is infinitely less; the strands of thread are straight, transparent, colored like the material, with an external aspect like a tree trunk with its bark.

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**Examination of colored cloth stained with semen.** This fabric, of a rose background, with small points and flowers of all colors, shows no appreciable stains, but in certain parts it is firm, almost starched, whereas there is a lot of suppleness in neighboring parts.

Several strips are cut out of the firmest portions of the fabric. Maceration and moderate rise in temperature of the liquid do not alter the color of the fabric, but it loses its stiffness, becomes less rubbery, so to speak, and an opaline deposit forms at the bottom of the capsule; a drop of alcohol makes the liquid quiver and it recaptures its transparency.

On examination by microscope, complete spermatic animalculi are clearly distinguished; the strands of diversely colored thread are identified by their volume and their particular appearance.

**Examination of fabric of cotton stained by semen.** One of these fabrics, a twill of blue cotton, shows a more intense hue in the stained parts; the stains are whitish, shiny, rubbery, stiff to the touch.

The addition of alcohol to the maceration mixture is enough to cause the distinct appearance of zoosperm and prostatic monads.

The other reagents have the same action here as in all the experiments we have already reported.

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This twilled fabric has the particular characteristic, that it is composed of a few strands of thread for the weaving and of cotton for the rest of the material.

On examination by microscope, the different nature of these substances are clearly distinguished. The thread has

the characteristics I have already described: it is straight, stiff, broken almost in splinters at the extremities, with the appearance of a tree trunk. The cotton is wound around on itself, twisted, so to speak, gathered into itself, its extremities clearly broken. Moreover, there is a multitude of small fibrils in the liquid, which are not seen in the maceration mixture of thread material.

Whatever the color of the strands of cotton, this distorted form, undoubtedly from the mode of spinning, is always seen.

I will not report all the experiments I have done on fabrics of cotton of varied colors; these nuances do not hinder the identification of spermatic animalculi.

**Examination of materials of wool stained with semen.— Examination of a piece of white flannel stained with semen.** There is no perceptible change of color on this fabric, and the stains are not appreciable to touch; instead of it feeling velvety, the fingers feel a sensation of rough dryness. In addition, the flannel is stiff at these points.

These stains, treated according to recommended procedures, furnish zoosperm on examination by microscope, as well as prostatic monads and a multitude of colored corpuscles.

The strands of wool are recognizable by their canalicular form; some of them do not have exactly the same diameter throughout, their surface is sort of wrinkled. In all, the strands of wool have a lot in common with hair, except that their volume is two to three times less considerable.

I also obtained satisfying results in examining sheets of various colors and fabrics of mixed wool and silk. 165

**Examination of dried seminal stains on material of silk.** I was able to get silk fabric stained with semen or vaginal mucus after coitus. I am going to report a few of the experiments I have done on this subject.

**Examination of a fabric of silk called foulard, of a violet and red color.** There are, on one of the faces of this fabric, stains of a greyish appearance, very shiny, of which there is no trace on the opposite surface; the material is stiff and starched in the stained parts.

These stains were macerated in distilled water which had been very gently heated; the solution turns violet. Some strands of silk detached and reached the bottom of the capsule, as well as flakes remaining suspended for a certain time.

Ammonia, sodium phosphate and alcohol equally cause the dissolution of seminal mucus and zoosperm then appear.

Filaments of silk cannot be confused with cotton or thread, for they resemble transparent tubes, having the same diameter throughout, but with no canals, and they have a volume seven to eight times less than hair.

I successively examined *satın* and *velvet*, which had been stained by semen or by vaginal mucus after coitus; I always succeeded in confirming the presence of spermatic animalculi.

I must remark that examination of velvet thus stained 166.

requires a very long maceration time and avoidance of its folding on itself, for more difficulty will be encountered in dissolving seminal substances. The use of sodium phosphate, as well as of alcohol always succeeded quite well for me.

### Summary of the Principal Facts of This Memoir

1) Spermatozoa conserve life and mobility as long as the mucus in which they swim remains fluid and warm. I have observed them living for ten hours: they die and rest imprisoned as soon as the mucus agglutinates.

2) The dried semen swells, disseminates, and divides in distilled water and in cold common water. It dissolves a little upon gently heating the maceration liquid and spermatic animalculi, characterized by their long tail, are seen with the microscope.

3) Dried semen dissolves in saliva as well as in urine and the animalculi are not altered.

4) Dried semen does not dissolve in blood or milk, unless diluted by a few drops of distilled water.

5) Alcohol or concentrated sodium, potassium, and ammonia solutions do not dissolve seminal mucus: they provoke its contraction and destroy the animalculi. On the other hand, these reagents have a very remarkable dissolving action if diluted with distilled water, in proportions variable for each of them as we have recommended.

6) To identify dried seminal stains on linen, and benefit from observation by microscope, care must be taken not to crumple or separate the macerating strips. In filtering the liquid of maceration, and examining the deposits left on the filter, the presence of complete spermatic animalculi, without tail breakage and isolated from mucus, is confirmed.

7) The presence of zoosperm in vaginal mucus, collected after the act of coitus between two slides, or dried on linen, is easily confirmed.

8) In women not affected with morbid discharge of the sexual parts I have always been able to find spermatic animalculi on linen or slides used to wipe the vaginal walls eight, ten, and even seventy-two hours after coitus.

9) On linen stained with semen and dried two months, one year and nearly three years before, I identified whole, complete zoosperm by their long tails.

10) The nature and color of the material stained by sperm does not hinder microscopical analysis and the confirmation of animalculi; they are identified as well on fabric of thread or cotton as on wool or silk.

11) Microscopical examination permits the distinction of the very different characteristics presented by filaments of flax or hemp, cotton, wool or silk.

### References

1. Du sperme, considéré sous le point de vue médico légal. *Journal de chimie médicale*, t. III, p. 469, 1827.
2. *Archives de médecine*, December 1838. Nouvelle application du microscope dans les expertises médico-légales.
3. Without speaking of the work of Devergie, in which this author announced his discovery of spermatozoa in the middle of maceration liquid of old semen stains, a work Bayard could not have known, since it was only published in our issue of January 1839, even though remitted to the committee in September, 1838, a note is found on the same subject, inserted by Ratier in the March, 1837 issue of *Journal de chimie médicale*. In macerating linen stained with semen in a watch glass, and submitting the liquid to microscopical inspection, this physician succeeded in finding spermatozoa; he pointed out in this regard the advantages legal medicine can derive from this mode of investigation. (*Editor's note*).
4. I have begun my research on this curious subject (April 10, 1839).
5. *Journal de chimie médicale et de toxicologie*. t. III, p. 469; October 1827.
6. A watch glass is preferable to every other capsule of a different substance, because the transparency of the glass permits examination by microscope of the deposit which forms after dessication; furthermore, watch glasses heat very rapidly. A glass dish would be still more useful, for the plane surface renders the examination easier.

## Observations on Some Reactions Shown by Semen Stains, Albuminous Stains and other Analogous Stains\*

J.-L. Lassaigne

405 The similarity of appearance, which certain albuminous stains dried on white cloth, show with seminal stains equally dried, can often confuse, on inspection, the first with the second. It is the same for white material stained by paste, starch, gelatine, gum or dextrin. The parts stained by these various substances present, in regard to certain physical considerations, the appearance of false seminal stains whose analysis and examination by microscope permit establishment of a clear distinction.

Called upon in several instances to determine the nature of various stains deposited on sheets and shirts after indecent assaults, we found that certain chemical reactions, produced on the stained fabric, can orient the examination and add new elements of proof to those invoked in this type of medico-legal assessment.

The tests undertaken by us showed that a drop or two of a potassium plumbate solution applied on an *albuminous stain* provoked a *fallow yellow* color bordering on brown café au lait after a contact of eight to ten minutes at a temperature of +20°. This effect was *not at all* produced either on seminal stains nor on *any other stain devoid of albumin*, such as dried stains of *gelatin, paste, starch, gum, or dextrin*.

The color displayed on albuminous stains on white linen is due to the formation of lead sulfide at the expense of sulfur, *a constitutive part of albumin*, as has been known for a long time. If a seminal stain, or a stain of another nature, has been deposited on white woolen material, the reagent concerned can develop a color, but only at the expense of sulfur contained in the wool. Thus, this reaction should not be attempted on white material made from this last substance.

In pursuing this work with other chemical reactions applied directly *on the parts of the material stained by the above-cited substances*, we determined different effects between albuminous and seminal stains. These results alone, however, cannot be called upon to establish positively the nature of the stains being examined, but they serve as useful accessories to the scientist when it is feasible to do multiple tests on the stains.

Among the chemical reagents which we used in our research, we will mention: 1) potassium copper subtartrate which, applied on semen and albuminous stains, color the

first in *bluish grey* and the second in *pale violet*; 2) ferric sulfate which communicates a pale yellow tint of rust to the semen stain and a reddish yellow tint to the albuminous stain; 3) *gold chloride* reacts more intensely on the albuminous stain and imparts to it a darker *ochre yellow*; 4) silver nitrate blackens the albuminous stain in diffuse light in less than a few minutes while the seminal stain, under the same circumstances, presents a *weak grey hue*; 5) mercurous nitrate behaves as the preceding nitrate, but much less energetically; 6) mercuric nitrate, under the same conditions of light and temperature as mercurous nitrate, exerts no action on seminal stains and causes the albuminous stain to pass to a *pale citrine yellow*; 7) cupric sulfate causes a *pale bluish grey tint* in the semen stain and a *deep sky blue* color in the albuminous stain; 8) finally, nitric acid at 40° causes the fabric stained with seminal fluid to become straw-yellow, whereas the same acid develops on the albuminous stain a *yellow* color bordering on *orange*. All the colors indicated above persist for a long enough time under diffuse light so that the comparative points between them can be established.

The gelatinous stains, those of paste, starch, gum or dextrin are in no way modified by potassium plumbate. As for the other reagents we tested on them, the effects are not appreciable enough that they can be characterized, and the results are not that clear-cut that a conclusion can be reached *a priori*.

We will add to the above-mentioned results another determination we have made in applying heat to the albuminous stains, as Professor Devergie first did on *seminal stains*. The caloric rays of incandescent coal, incapable from a distance of scorching the linen on which is found the stain, causes in these stains a *dark nankeen-yellow* color, whereas albuminous stains do not appreciably exhibit this color or do so very weakly.

This action of heat on seminal stains can be applied to white linen, on which the soluble part of the semen stain has dried. Here we should point out that we have already applied this type of test in an affair of an indecent assault which had been placed in the hands of Lesueur and myself. (The Léandri Affair).

By transferring the substance, soluble in cold water, extracted from an extensive seminal stain on a colored silk petticoat to a piece of white cloth, we could determine the *stiffness of the material and its coloring in pale yellow under the influence of a suitably moderate heat*.

\* Translation of: "Observations sur quelques Réactions que Présentent les Taches Spermatiques les Taches Albumineuses et Autres Taches Analogues." in *Annales d'Hygiène Publique et de Médecine Légale* 10 (2nd series): 405-408 (1858).

The facts presented in this report find their application in various circumstances: thus, in publishing them today in *Annales d'hygiène et de médecine légale*, we intended to do a service to all those occupied in analogous work, and desiring to control such work with experiments.



## Seminal Stains and Blood Stains\*

**D. Cauvet**

Professor of the École  
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On June 8, 1874, we were charged with "examining the shirt of a person named Zohra ben Ahmed and the "gandoura" [a long sleeveless shirt] of a person named Brahim bel Belkassem, to investigate by all possible means whether these articles of clothing bore seminal stains, and to say, further, whether the gandoura of Brahim ben Belkassem bore bloodstains as well." We received two packages, labelled as follows:

**Shirt of Zohra ben Ahmed (victim)**

**Gandoura of Brahim ben Belkassem (accused of rape)**

1. **Examination of the shirt of Zohra ben Ahmed.** This shirt is made from two pieces of cotton cloth sewn crosswise; it has no longitudinal seam; the two upper pieces are attached to a third near a border, such that there are two unequal openings: a smaller one, for the left arm, a much larger one for the head and the right arm. Made in this way, the piece of clothing was meant to be suspended from the left shoulder, leaving open the left arm on one side, and the head, right shoulder and arm on the other.

The absence of longitudinal seam allows the body to be partly uncovered in certain kinds of movement.

We described the shirt of Zohra assuming that the opening was in front; it is probable, however, that the shirt was worn front-to-back, that is, that the longitudinal opening was in back. This fact might be one of the reasons for the rape. The verbal statement of the doctor stated that Zohra was bent forward, and that she had been seized from the back. If, in this bent-over position, Zohra was only little-clothed, as the facts seem to indicate, if the shirt was simply tied at the waist with a sash, then the greater part of her body was uncovered, especially on the left side: the view of Zohra's form, which the doctor's report said was large and well formed, excited the desires of the one who committed the violation.

The shirt is 1.2 meters long; the material which makes it up measured 2.7 meters along its lower edge. This shirt is extremely filthy, covered by all sorts of stains, mainly stains tinged with blood, generally stiff and starchy, here and there brown with light clots, here and there greyish-red.

The stains occupy the entire width of the material, from its lower edge to the level of the genital organs. On various

parts of the shirt, particularly the side which would be worn in front, were noted a large number of green stains, apparently of cow dung.

The widespread stains on the garment are in such quantity that it was impossible to go into a detailed description. In the examination which we conducted, we divided them arbitrarily into groups, according to our best judgment, not every group containing the same number of stains.

Stain No. 1. Located at the uppermost right corner; composed of three masses of brown material, enclosing some fibers of white wool, and apparently formed by a large, dried clot (No. 1).

Stains No. 2. 15 cm from the lower edge and 30 cm from the right edge, a group of stains, pale red, elongate, generally directed from bottom to top and from left to right; one of these, sufficiently dark, to show at its apex a rounded, greyish, starched stain (No. 2).

Stains No. 3. Above group No. 2, reddish-grey stains, some of which are paler than the others.

Stains Nos. 4 and 5. Very large irregular, quadrilateral stain, directed obliquely from left to right and from bottom to top, with a width of 20 cm. length of 28 cm, and diagonals of 35 and 33 cm, extremely pleated, stiff, crinkled, with some clots at the left upper corner, and especially the right lower corner; paler and greyish red on the upper, red brown on the lower, where it appeared formed by blood and vaginal mucus.

This stain is extremely starched toward the middle of the left edge and the middle of the right edge, where it seems to be constituted by a mixture of blood and semen (No. 3).

Three strips of fabric were removed: 1) from the lower part (no. 4); 2) from the left (no. 5); 3) from the right (no. 6).

Stains No. 6. To the left and below stain 4-5 numerous stains, reddish grey or pale red, sometimes a bit tinted in green, of variable size; some comprising simple drops; others, somewhat large, showing that Zohra wiped herself after coitus (no. 7).

Stains no. 7. To the left of stains no. 6, a group of stains of the same form, larger, more numerous, a somewhat deep red, extending from top to bottom up to 60 centimeters from the lower hem (no. 8).

Stains no. 8. Groups of stains: two superior, juxtaposed, on the whole, 34 centimeters long, 18 centimeters wide, especially colored on the lower part, where they presented the

\*Translation of: "Taches de Sperme et Taches de Sang." in *Annales d'Hygiène Publique et de Médecine Légale* 44 (2nd series): 117-126 (1875).

appearance of trails of blood, and red brown encircled in grey. Below, various stains, 10 centimeters from the hem and appearing, for the most part, due to splatterings. Between these two groups of stains appeared stains formed especially by drops of blood, the greater part stiff and extremely starched.

Stains no. 9. Group of disseminated stains, somewhat deep red brown.

Stains no. 10. Twenty centimeters from group 9, a not very apparent stain, brownish grey, starched, 25 millimeters long, 1 centimeter wide (no. 9).

Stains no. 11. A large stain situated on the lower hem, 30 centimeters from the left hem, irregularly triangular, 20 centimeters wide, 12 to 15 centimeters high, crinkled, deep red brown, with small, disseminated clots, apparently formed by blood and vaginal mucus.

Stains no. 12. Above the stain no. 11, a group of red stains, one of which is central, larger, red at its periphery, from which escaped bloodied trails, greyish yellow, with a sort of grey crust in the middle (no. 10).

This grey matter treated with preserving liquid released carbonic acid. It appears constituted by lime carbonate (chalk), either alone, or mixed with starch.

Stains no. 13. Above stains 12, and about 25 centimeters from the left hem, a group of stains seeming to form but one, 18 centimeters wide, 12 centimeters high, pale red in its central parts, greyish toward the outside. The median covered in places by greyish crusts (no. 11).

Stains no. 14. A large triangular stain with the left hem as its base, 22 centimeters wide, 20 centimeters high, crinkled, stiff, a reddish grey brown (no. 12).

Attentive examination of the stains existing on Zohra's shirt showed that these stains are extremely numerous and widespread over the whole area of the fabric at the level of the genital organs.

They can be arranged in three categories according to form and nature.

With regard to form, they are: 1) very large plaques coming from blood flow effected after coitus; 2) smaller stains resulting from application of the fabric to the vulva; 3) drops of blood.

With regard to their origin, or better, their nature, they appear due 1) to almost pure blood; 2) to blood mixed with vaginal mucus; 3) to blood mixed with semen.

It seems then that the aspect and position of these stains should permit the affirmation that Zohra ben Ahmed was the victim of rape, and that this rape was consummated not too long ago for the blood stains still showed small clots in several locations. These stains cannot be attributed to any other cause, the medical report confirming that the victim has not yet menstruated, and that her body presented no trace of violence, with the exception of the external portions of the sexual organs.

The preceding considerations, however, can allow only presumptions about the nature of the stains. Only examination by microscope of those of the stains which seem the

best characterized can furnish precise information in this regard.

**Examination by Microscope.** In stains resulting from rape, the elements to be sought on the shirt of the victim originate from two sources:

1) the perpetrator of the rape; 2) the genital organs of the victim.

If the rape was committed with violence and especially when the sexual organs of the woman are not yet sufficiently developed, a somewhat large amount of blood will be added to material of purely sexual origin.

The elements originating from the perpetrator of the crime are corpuscles normally found in semen which detached them and carried them along during its passage. These are: spermatozoa, spermatic cells, epithelial cells of the urethra, or the epididymis, etc.

The elements originating from the sexual organs of the young girl, other than blood resulting from the tearing of certain parts of the vulva are corpuscles of the vaginal mucus and epithelial cells of the vaginal wall.

To arrive at a determination of these elements of such diverse nature we chose those stains appearing most characteristic to us, as we have noted above.

A strip of material was removed from each of these parts with scissors and placed in a watch glass, then a few drops of preserving liquid of Roussin were added.

At the end of one or two hours, according to the condition of the stain, the strip of material was dissociated and some of the liquid it had soaked in was removed by expression with a glass pipette. Finally, one of the threads was placed on a slide and carefully unravelled, so as to isolate the filaments. Some of the liquid separated with a pipette was then added, and everything brought to the microscope.

After meticulous observation of the preparation thus obtained, a drop of iodine solution was added, and the examination was once again performed.

The first observation had as its purpose the determination of epithelial elements, mucus cells and blood corpuscles, i.e. histological elements, whose color and special form permit an easy determination.

The second observation was to furnish a means of easy recognition of spermatozoa, whose transparency and lack of color render them difficult to distinguish. On contact with a solution of iodine, on the contrary, these organelles take on an evident relief and stand out clearly.

Each preparation, then, was the object of two successive examinations.

Before the presentation of the results obtained, it should be pointed out that, among the microscopic elements observed, we noted only those whose presence offered some interest.

Here are the results:

1) Stain no. 1.—Matter of a mucus nature, without well-defined blood corpuscles, with numerous groups of *merismopodiae*.

The origin of this matter remains unknown, nothing in the

constitutive elements being able to furnish information in this regard.

2) Stains no. 2.—Blood corpuscles; leukocytes; epithelium (uterine?); epididymal cells (??); no spermatozoa.

3) Stains no. 3.—Several spermatozoa.

4) Stains no. 4.—poorly defined blood corpuscles; no spermatozoa; cuboidal epithelium; cylindrical epithelium of the uterus (?) or the epididymis (?);

5) Stains no. 5.—Blood corpuscles; questionable spermatozoa.

6) Stains no. 5.—Blood corpuscles; spermatozoa.

7) Stains no. 6.—Blood corpuscles; no distinct spermatozoa.

8) Stains no. 7.—Blood corpuscles; no spermatozoa.

9) Stains no. 10.—One single spermatozoan seen.

10) Stains no. 11.—Blood corpuscles; a spermatozoan.

11) Stains no. 13.—Spermatozoa; blood corpuscles; vaginal epithelium; urethral (?) epithelium; epididymal (?) epithelium.

12) Stains no. 14.—Blood corpuscles; cylindrical epithelium; vaginal epithelium; spermatozoon.

The results just cited permit the presentation of the following conclusions:

1) The shirt of Zohra ben Ahmed is stained with blood and vaginal mucus;

2) Stains nos. 3, 5, 10, 11, 13, 14 are formed by blood mixed with semen;

3) Zohra ben Ahmed was probably the victim of a rape.

2) **Examination of the gandoura of Brahim ben Belkasssem.** A white woolen shirt, having one single seam, situ-

ated toward the middle of the shirt and directed from top to bottom. This shirt is 88 centimeters long, 30 centimeters wide; used, filled with holes and tears; a part of the front left shirttail is missing. It presents only one group of stains located a bit below the waistline, 6 centimeters from the seam and 23 centimeters from the lower hem.

These stains, 5-6 in number, are brown, rather small, a bit stiff. They do not pass through the fabric. The two largest are: one, to the left, 2 centimeters long, 15 millimeters wide; the other, to the right, triangular, 3 centimeters high, 3 centimeters wide at the base.

These stains were submitted to the treatment indicated for those of Zohra's shirt, and the preparations thus obtained were carefully examined by microscope.

This research having brought no results, the presence of blood was sought for by chemical means, but it was impossible to obtain any of the characteristic reactions or to obtain production of hemin crystals.

These negative results permit us the presentation of the following conclusions:

1) The stains observed on the gandoura of Brahim ben Belkasssem are not blood stains and contain no semen.

2) The position of the stains, moreover, causes difficulty in the conclusion that they were produced by blood coming from the sexual organs of the victim.

3) If Brahim ben Belkasssem is the perpetrator of the crime ascribed to him, it is not on his gandoura, which is too short, that traces can be found, but on the garment he undoubtedly wore over this gandoura.

## On Seminal Stains\*

Professor Brouardel

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From a Course in Legal Medicine

332 An important question, and one which is often submitted  
for evaluation by an expert physician, is that of the existence  
of seminal stains. We will not be paying particular attention  
to the description of the procedures or chemical research  
333 formerly employed, their utility and value having disap-  
peared with the discovery of the existence of the sper-  
matozoon, which is the essential, characteristic element of  
semen. They [the stains] can be of highly divergent dimen-  
sions, their tint is a yellowish grey, their form irregular of  
sinuous contour like a geography map, their edges pre-  
sented a deeper shade than the central parts. To examine  
them well, it is necessary to look at them not only directly,  
but also by transmitted light. It is necessary to do this as  
much as possible, not with sunlight, but with diffuse light,  
filtered by clouds. If it is a matter of experimenting on linen,  
the impregnated places are much more transparent and per-  
mit a better view of the weft of the fabric.

They are most often to be identified on shirts, linen and  
fabric. In young girls who have been raped, Devergie  
thought they could be found especially in front. This opinion  
is too absolute, and it is recommended that they be looked  
for everywhere; they can even be found on the sleeves near  
the armpit. If it is a matter of a young boy, it is necessary to  
note whether they are found on the anterior or posterior part  
of the shirt. When found on the back part, it is important to  
see if they are mixed in with fecal matter, indicating the  
practice of pederasty. Their dissemination has been consid-  
ered a proof that the young girl had struggled, but it could  
also be a matter, on the contrary, of repeated venereal acts.

In the Cr. . . . affair, for example, a case of this elderly  
woman murdered by young people, to whom she frequently  
surrendered herself, there were three beds, one of which  
served as the seat of activity. It was the only one which was  
ordinarily used. On its surface were no less than seventy  
seminal stains, some of which were not less than 10 to 15  
centimeters long. The preliminary examination required de-  
termination of whether it was a matter of habitual inter-  
course, or if the numerous stains could result from repeated  
acts, recently consummated, during the last 24 to 36 hours.  
It is easy to understand that a categorical response was  
impossible.

When there is an assessment to be done on stains and the  
stained parts of the fabric are cut out, it is good practice to

number each strip, such that, if necessary, they can be re-  
turned to their place, reconstituting the sheet or linen in its  
entirety, which permits saying: there is a seminal stain, here,  
one of mucus, etc. In general, linens submitted to experts are  
334/ soiled with stains of every type, from which the useless ele-  
ments must be eliminated. In addition, an important point to  
know, from the personal point of view for the physician, the  
shirts and sheets remitted to him frequently contain numer-  
ous varieties of parasitic insects, seeking the opportunity to  
multiply. It is thus prudent not to let these pieces get into his  
home to avoid this invasion.

It can be necessary to look for seminal stains on sub-  
stances other than linen, for example, on colored fabric, on  
various objects and, finally, on the body of the victim. Thus,  
this very morning, I had to look for seminal stains on the skin  
of the corpse of a little girl. There were some above the pubis  
and on the upper part of the thigh. In these cases, a shiny  
stain is discovered, especially if regarded with certain inci-  
dences of light. It is generally rather easily separated from  
the superficial layer of epidermis for examination. Semen  
must also be sought in the vagina, the uterine cavity, and the  
fallopian tubes. Sperm enjoys the property of remaining  
alive for a rather long time in natural cavities. But when the  
vaginal mucus is acid, or when it becomes so by alteration,  
it provokes the destruction of spermatozoa.

On the contrary, in the interior of the uterus, where  
the secretion is alkaline, sperm is much better preserved.  
Dumas, the first to describe spermatozoa, found some, living,  
in the ovaries of dogs seven days after mating. In an autopsy  
it is then necessary to go further than a superficial exam-  
ination and to pursue intently the search in the uterus, fallo-  
pian tubes and ovaries.

If the assessment is done soon afterwards, the movements  
specific to spermatozoa can be made to reappear in moist-  
ening linen soiled with sperm, and characteristics analogous  
to those presented by fresh sperm can thus be found. What  
then are these last-mentioned characteristics? According to  
Robin and the latest research of micrographers, a spermat-  
zoon is a simple cell furnished with a very long prolongation  
which is nothing other than a strong vibratile cilium. What-  
ever it might be, this vibratile cilium communicates very  
clear movements to the rest of the cell, which seems to direct  
itself and have an instinct like a true animal. The total length  
of a spermatozoon is 45 thousandths of a millimeter or  $\mu$ . Of  
335/ this figure, the head is only about  $5\mu$  in length, 3 in width,  
and 2 in thickness. In addition, leukocytes, round fatty

\*Translation of: "Des Taches Spermatiques." in *Le Practicien* 2 (28):  
332-336 (1879).

bodies, other small bodies called "*sympexions*," and tribasic phosphate crystals are found in semen. It is quite rare that whole spermatozoa can be observed. Most often, linen brought to the expert has undergone changes and crumpling which have most often separated the heads and tails such that only debris are found.

Along with spermatozoa, elements derived from the environment from which the sperm comes can be seen. Thus, in the affair of Mme. Cr . . . , a handkerchief was found on which were numerous stains perfectly analogous to those of semen. Examination by microscope permitted discovery of round, elongated cells, epithelial cells of the mouth, other globular cells of mucus and, finally, cylindro-conical cells of the respiratory tract. It was probable then that at a given moment the semen found itself in the mouth. This hypothesis was strongly confirmed with the discovery of a grain of tobacco mixed in, which left no doubt as to what had happened, the murder victim being the only one who indulged. If there had not been tobacco grains, there could have been a hesitation in distinguishing between cells of the respiratory tract and certain other cells with vibratile cilia found in the epididymis and mixing with semen.

In the frequent cases where there is a mixture of *vaginal mucus*, large, unequal, rolled-up epithelial cells, mixed in with a certain number of pus corpuscles, are found. Leukocytes become predominant only if there is *vaginitis*. *Fecal matter* is recognizable due to the presence of *twisted fibers* coming from poorly digested muscle tissues, numerous grains of starch and vegetable cells.

Finally, epithelial cells coming from urethral mucosa and epidermal cells with very elongated vibratile cilia are also found mixed in with the sperm. These latter cells are generally abundant in semen of the first few penetrations; thus, for example, they appear only after a continence of about ten days and disappear with repeated coitus on the second or third time.

What is the best procedure to be followed for systematic examination of seminal stains? On dried linen, which had been folded several times besides, and carelessly treated, it is common to see the seminal stain partially peeling off in scales. It is necessary then to collect these separated parts carefully and moisten them slightly with water. But ordinarily, none of the stain can be separated dry, and it is necessary to use one of the following procedures: after strips of contaminated linen have been cut out, they are left to macerate in two or three drops of distilled water, placed in a watch glass. The amount of water must be as small as possible, in order to examine all the parts easily. Another method, attributed to Robin, is to suspend the strips above water in glass test tube such that the strips touch the surface of the liquid, which will imbibe it by capillarity. These procedures for renovation of the seminal stains are equally good, with the condition that one has the time, for 12 to 15 days are sometimes necessary to permit inhibition to go to completion. Once obtained, the surface of the linen is scraped with a scalpel, the threads of material are detached one by

one and examined by microscope.

These procedures are applicable when strips or thongs of fabric can be detached with scissors. But this is not always possible, it being either a matter of priceless furniture, or of the stains being located on the skin, for example. These stains are softened by moistening, and scraped however possible. Sometimes the search for spermatozoa is rendered more difficult by the considerable number of epithelia, grains of starch, etc., which clutter the preparation.

It is essential to examine each stain in its entirety. When spermatozoa are not found after examination of the greater part of the stain, one must not give up on their discovery, for they are often found collected in one single point. It is also necessary to develop the habit of always using the same magnification, that of 500 diameters, for example; it is easier then to recognize the presence of spermatozoa. With the same goal in mind, Kasper recommends leaving the liquid presumed to be seminal to dry between the two slides of the preparation, then to remoisten it a little while afterwards. In proceeding thusly, it seems that air fixes to the walls of the spermatozoa and increases the clarity of their contours. Roussin also recommends coloring the preparation with a drop of tincture of iodine.

Even when spermatozoa are not found in a stain having all the macroscopic characteristics of a seminal stain, the conclusion that it is not actually seminal cannot be reached. Indeed, there is a certain number of individuals who are infertile, during a certain time at least, due to an absence of spermatozoa after a double orchitis, for example (as demonstrated by Professor Gosselin). They nevertheless ejaculate a fluid showing every appearance of normal semen. It has also been proposed that spermatozoa disappear in the elderly. This is often not the case, and Dr. Duplay, Jr., has determined their presence in elderly men, 85 years old. Dr. Dieu, a physician of the disabled, has found in autopsies that, after the age of 70, only a quarter of the subjects examined no longer possessed spermatozoa, whereas the remaining three quarters had them. Finally, if coitus has been repeated a number of times, the later penetrations furnish a semen lacking in fecundating properties. Nor will spermatozoa be found if the fabric, on which the investigation is being performed, has been energetically crumpled; it is, therefore, necessary to wrap and fold it carefully.

There are a certain number of cases where distinction must be made between seminal stains and those of gonorrhea. This latter gives greenish stains at its outset, then yellowish and finally uncolored. Their surface area is much smaller than that of seminal stains, their form round and more regular; they are sometimes colored by the coloring matter of blood and contain a large number of leukocytes. The interest in this distinction can be imagined if it is a matter, for example, of a young girl pretending to have been violated, and whose shirt presents stains of gonorrheal mucus.

Thanks to examination by microscope it is almost impossible to confuse spermatozoa with anything else or semen

with any other liquid. However, Hoffmann has pointed out the possible confusion of the spermatozoon with certain bacillary bacteria, also formed by a head and a vibratory cilium, but this latter is ten times smaller than the spermatozoon, and this error will be avoided if the recommendation given above, that of always using the same magnification, is followed.

## Report on the Petel and Labiche Procedure for the Detection of Seminal Stains\*

Boutmy and P. Brouardel<sup>1</sup>

/224 Dr. Brouardel and myself were charged by the Society of legal medicine with examining a work resulting from the collaboration of Petel, an M.D., and Labiche, a pharmacist, a work relating to the use of carmine in the medico-legal examination of seminal stains.

I will communicate to you the conclusions to which the study of this work has led us.

When it is a matter of animal secretions, the experiments it is important to perform to enlighten justice are of two different types, namely:

Chemical experiments;

Anatomic experiments.

/225 When both of these types of experiments can be performed, it is possible to arrive at an unequivocal answer to the question; but when, by fortuitous circumstance, one of the two types of experiments cannot be performed, the expert hesitates to assert his opinion absolutely.

This hesitation is understandable, especially when it is the anatomic study which is lacking.

In fact, the chemical reactions which can serve to characterize the liquids of the organism are very restricted in number and limit themselves generally either to coagulations by heating or by a few reagents such as nitric acid, mercuric chloride, phenol and wine alcohol, or to various colorations which certain substances cause to appear in the material being examined.

As a result, the chemical reactions we have just presented, applied to the study of the organism, indicate the class of material rather than the particular identity.

In contrast, in the presence of the anatomic element (which is always unique), confusion is impossible, and the expert can give an opinion with every assurance.

Because of blood cells, for example, blood will not be confused with any other liquid of the organism; and, in addition, every anatomist can distinguish mammalian blood from that of bird or fish.

Further, as with blood, spermatic liquid contains this particular organism, *the spermatozoon*, imprinting it with a very special trademark, and permitting its absolute differentiation from every other liquid of animal origin.

As a result, when one or several spermatozoa can be ex-

tracted from any stain whatever, this stain contains semen. A spermatozoon can always be recognized by its very distinct form; and it is precisely this which gives this anatomic element its extreme importance in research in legal medicine.

But, as many authors will note, it can happen that, after crumpling of the material to be examined, the spermatozoa break into fragments, difficult to recognize, and that, as a result, the anatomic examination by microscope will not lead to any precise conclusions.<sup>2</sup>

It is this kind of regrettable circumstance, continue Petel and Labiche, that we would like to avoid by pointing out that carmine can impregnate seminal stains with a special rose color, resistant to washing of the fabric, and to the action of certain reagents.

This method of investigation, proposed by Petel and Labiche, is bound, we believe, to be very useful, and the authors must be congratulated for the care with which they carried out the studies, and for the skill with which they removed causes of error which can arise from the presence in the stained material of elements produced by saliva, nasal mucus, albumin, etc.

But in our eyes, it would be exceeding the limits of discretion to agree with Petel and Labiche that, in absence of spermatozoa, the persistent red coloration, communicated to stains by carmine and remaining on the material, is sufficient to decide on the presence of semen, especially since this fact can engender such serious consequences.

Indeed, in imagining the terrible sentence which a man might receive for the crime of rape, what expert would dare assert that a stain found on material is a seminal stain because this stain colors in rose by the action ammoniacal carmine and then takes 12 hours to discolor in a sodium-carbonate bath, whereas another stain, produced for example by albumin, will discolor in six hours.

Could not a particular circumstance, yet unknown to the authors of this memoir, occur where this stain of albumin disappears only after 11 hours, for example?

Is it absolutely certain that among all the stains which can be formed on fabric, no type will be found which shares with seminal stains the double property of coloring in red with carmine and also resisting alkaline solution for 12 hours?

This 12-hour limit for sperm stains appears to us very arbitrary, and if it diminishes, what will become of the investigative procedure using carmine. In these circumstances, the anatomic element remains the only absolutely certain sign of the presence of semen; and if it is sometimes found

\*Translation of: "Rapport sur le Procédé Petel et Labiche Destiné a Faire Découvrir les Taches de Sperme". in *Annales d'Hygiène Publique et de Médecine Légale* 4 (3rd series): 224-227 (1880).

<sup>1</sup> Meeting of May 12, 1879.

<sup>2</sup> It is also known that the seminal fluid of certain individuals is devoid of spermatozoa.

that spermatozoa are absent, these cases are so rare that the public conscience cannot be noticeably stirred over it.

It will be agreed that, in a comparable circumstance, it is necessary to be very circumspect, and that it is better to acquit the guilty than to condemn the innocent.

Does this mean that the method of investigation proposed by Petel and Labiche must be rejected? As we have mentioned, we think the contrary, for it brings an additional

proof to that already obtained by microscope. This accumulation of proof is always desirable in research as delicate as that with which we are occupied. We thus propose that the society express its gratitude to Petel and Labiche for this presentation of a new reagent, confirming the histological results obtained in the medico-legal investigation of seminal stains.



## Memoir on a Few New Applications of Microscopical Examination to the Study of Different Types of Stains \*

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/416 Called upon of late to give our opinion on many cases of legal medicine of great interest, we have had the opportunity to do some new applications very important in the examination of different types of stains. We are eager to include here the principal results which can be useful to other experts and which add to what we already know about the correct procedures for identification of the nature of the substances of which we will be speaking. Additional proof of the superiority of the microscope over ancient methods in the medico-legal evaluation of all types of stains will be found in this study.

### Blood stains

**Is the stain submitted to an examination by experts from the blood of a man or of a woman?** It is not uncommon to see experts called upon to answer a question posed in the same form as that which serves as title of this section. Just such a question was posed in a rogatory commission, in the execution of which we had been committed to examine different articles of clothing, stained with blood from the murder of an elderly man and his domestic, an elderly woman.

/417 There have been only a small number of research publications on this question; the treatises of legal medicine lack documentation which might serve to answer it; which is why we thought it might be useful to publish the studies we have done in the circumstances we have just briefly mentioned.

Although by the manner in which the question is posed: "What are the blood stains present on the smock, etc.?", there is no doubt cast on the fact that stains are formed from blood, the experts ought to have assured themselves beforehand as to their exact nature. After having noted that they have the physical characteristics of blood stains, we confirmed with the aid of appropriate microscopical and chemical procedures, that many of the spots actually contain red blood cells, white blood cells and fibrin, the essential characteristics of blood.

Our observations and analyses ought to have been directed principally toward the particular question as to whether the stains present on the smock were formed from the blood of man or the blood of woman.

Knowing that experience has shown that the character-

\*Translation of: "Mémoire sur Quelques Applications Nouvelles de L'Examen Microscopique de Diverses Espèces de Taches". in *Annales d'Hygiène Publique et de Médecine Légale* 13 (2nd series): 416-442 (1860).

istics which enable a distinction of male blood from female blood must, to be confirmed, even when the quantity of material is sufficient, be investigated as soon as possible after the blood leaves the vessels, and that the objects alleged to be stained with blood and furnished for our examination, were remitted to us twenty-one days after the crime, we immediately conducted a special study of the stains present on the smock in the following manner.

After having removed the portions of material bearing the more important stains, we meticulously cut these to separate the portions of clothing stained and imbibed with blood from those which were not. The bloodied portions were reunited at the bottom of a short, wide test tube; we then moistened them with a little distilled water; the moistening finished, we added a quantity of sulfuric acid, concentrated, pure and uncolored, equal to about half the substance to be tested; having mixed and compressed everything together in order to render the action of the acid on the stained material even and complete, we tried recognizing the odor emitted from this substance. We noted a light odor of human sweat. Despite repeated attempts under the preceding conditions with gradual increases of the amount of acid, it was impossible to obtain an odor pronounced enough for the comparison of the odor presented by the blood of an elderly man with the blood of an elderly woman, treated in the same way, to give us conclusive results in either direction.

It is known, moreover, that, if a sufficient amount of blood or stains big enough and recent enough, treated as previously, give a particular odor for each animal species which experiment permits a distinction, this characteristic is not sufficiently pronounced for permitting verification that the blood comes from one animal rather than another. Only in the case where the examination has been conducted within an appropriate period of time, and this characteristic is lacking, is it necessary to suppose that the blood does not come from the presumed animal.

It is also known 1) that, if blood from man and blood from woman in sufficient amount or forming stains big enough and recent enough, treated as previously, give an odor comparable to human sweat; 2) that, if this odor is less strong or a bit more bitter in bloodied material coming from a woman than from a man, these characteristics become more and more analogous, then similar, with time. They are not even pronounced enough at any time, that, provided with such a small amount of material as had been submitted for our

419 examination, it would be possible to affirm with certainty that a human blood stain comes from one sex rather than the other.

In summary: the age of the stains, dating twenty-one days, submitted for our examination; the small amount, relative to the question to be resolved, of the bloodied matter which formed the stains; the natural and constant similarity between blood of man and of woman, which differ only temporarily and by weak degrees of a given odor, comparable to that of sweat, make it impossible for us to decide by the light odor of sweat emitted by these stains if it is the blood of man or the blood of woman which forms them; but nothing authorizes a denial that the blood comes from a person of feminine sex.

**A note on the distinctive characteristics, from a medico-legal viewpoint, of blood stains and stains from fly droppings.** In a medico-legal investigation, a smock bearing stains, allegedly blood, was submitted for our examination, for the purpose of determining if they actually contain elements characteristic of blood, to which they presented a superficial resemblance.

Near the lower border of this smock were three circular stains, of width from 1-2 mm, forming a thin glaze on the material which they did not saturate for the full thickness; they were of a reddish brown, fairly shiny on their surface and a bit stiff, almost starched. Studied according to standard procedure in search of elements of blood, we found no instance of them. They indisputably presented, on the contrary, the microscopic characteristics and the parts constituting fly droppings in all their aspects. Like these droppings and like the substance of stains which they form on furniture and material, they are composed of a material homogeneous, amorphous, transparent, uncolored, swollen, dissociated by, or dissolved in, water, holding together the coloring granules of these droppings. These granules formed, as always, the greatest mass of the material of the stain, in which they were almost contiguous. They were of a yellow brown, some with a greenish reflection, the others with a reddish reflection, faintly pronounced. They all strongly refracted light, clear at the center, dark on the periphery, as fatty bodies; also like fat granules, they were insoluble in water and in acetic acid and almost all dissolved in hot alcohol and in ether. Some small crystals in the form of short needles of undetermined chemical composition accompanied them.

These characteristics can be found, as one can easily be assured, on almost every fly dropping examined. This permits us to conclude that it is a matter, not of blood stains, but of stains formed by fly droppings.

**A medico-legal note on the stains of varnish which show all the physical or superficial characteristics of blood stains.** We were entrusted, on 16 December, 1859, to proceed with the analysis of stains present on the smock confiscated from the home of Mr. B. . . ., accused of homicide, and with determining: 1) if the stains are of blood, of smoke or other substance, 2) if they are of human blood or cow blood, 3) if they are recent, with regard to their existence, of fifteen

months, as the accused asserts, and that since then, the smock must have undergone two or three complete washings. And, before proceeding with the analysis, to examine the material of the stains, and determine: 1) if they had not been superficially washed, with the goal of making them disappear; 2) if they do not conserve a gummy nature inside the smock, indicating that they have not undergone any washing on the inside. 421

This smock was on the whole of blue cloth, a bit whitened from decay and use; especially on its exterior surface, the back as well as the front, on the sides of its slit. It was patched near the neck and on the sleeves.

*Stains offering the superficial aspect of blood stains.* On the right side of the smock, in front, on the chest, shoulder, the upper part of the sleeve on this side, and a little underneath the seam of the armpit, could be seen very small stains too numerous to be counted. There were also some, exactly the same, on the sleeve on the left side. They were of a width of 1-18 mm; the greater part were separated from each other; some were joined together at their ends. Almost all were round or oval; the others were polygonal, with rounded angles. All ended in a distinct border as dark as the rest of the stain. All traversed the entire thickness of the fabric and were as discernible on the side of the smock turned toward the body as on the outside. Most of the ones on the side of the smock turned toward the body presented an aspect slightly brilliant, gummy, that were found only on a small number on the outside surface. All gave to the fabric a stiffness, comparable to that which starch gives to shirts, and to that which blood stains and other sorts of mucus and albuminous liquids of the human body give to different sorts of linen. None formed a crust on any face of the smock whatever. All these stains presented a reddish-brown taint, slightly shiny or gummy on one side, as was just pointed out; an aspect similar to that which blood stains present. This reddish-brown color lost its reddish tint in all the stains located in the portions of fabric colored in dark blue; but the gummy aspect and the stiffness peculiar to starched cloth was still clearly evident. 42

These stains were evidently duller on the outside surface than on the side turned toward the body; the appearance which they presented in this context could be compared on the outside surface only to that which an incomplete washing, or even better, a scraping after dessication, gives to blood stains. On the side turned toward the body, these stains showed all the superficial characteristics shown by stains formed by blood, and, on the outside surface, the superficial characteristics of the same stains when they have been incompletely washed or rubbed and scraped without having been spread out.

One single physical particularity was lacking in them, it being that, in the obscurity of night, the light of a lamp and of a candle did not render the stains appreciably more shiny, nor more visible, while this occurs, on the contrary, for blood stains.

Nonetheless, their similarity to stains actually formed by

blood was such that we had to proceed with their analysis as one does in the case of stains strongly considered to be formed by blood.

*Microscopical and chemical analysis of stains presenting superficial characteristics of blood stains.* We cut out the stains and, according to the procedures known to science, plunged them alternatively either in a sodium phosphate solution, or in a sodium sulfate solution. These liquids, which should slowly swell and soften the substance of the blood stains, with the purpose of then isolating the constitutive elements under the microscope, had absolutely no effect on the stains. Water itself in no way modified them.

423 We then submitted the stains to prolonged immersion, then to repeated washings in cold water and in hot water, both pure and soapy. These washings changed nothing in the taint nor the starched state of the stains.

These facts sufficed at this point to show us the stains we were analyzing were in no way formed by blood.

The immersion and then the washing of many of the stains, done separately in liquid ammonia, in carbon disulfide, in alcohol and in ether removed the gummy aspect from the stains on the inside of the fabric; they caused the starched state to disappear, but not completely, and the mark of faded stains distinctly persisted on the two surfaces of the fabric. The evaporation of the alcohol, the ether and the ammonia in which many of the cut-out stains were immersed for eighteen hours, left a residue of only small amount, which presented no crystals. This residue dissolved in sulfuric acid, but not acetic acid. The small amount prohibited us from submitting it to the action of other chemical agents.

This resistance to the action of pure water, of soapy water, of ammonia, of alcohol, of ether, and of carbon disulfide, tends to make one admit that the stains could not be recent, could have an existence of fifteen months, and that they could have resisted two or three complete washings that the smock would have undergone since the time of their formation, but without it being possible to assign a date to their formation.

The absence of the gummy aspect of the stains on the outside surface of the smock with conservation of this aspect on the inside surface could be due to repeated rubbing of the stains and the wearing out of the smock, which had already modified the general color; but the resistance of the two sides of the stains to the action of water and of the chemical solutions we employed is in opposition to the theory that it might be due to a washing done beforehand.

424 The amount of substance coloring the fabric and giving it the starched condition was so small, that it was impossible for us to retrieve by chemical means an amount sufficient for precise determination of the type of resin, or of varnish, forming the stains. From this point, our only recourse was the use of the microscope to see what type of material was present.

We proceeded to the microscopical examination of the material of the smock, and of the substance forming the stains,

examining it between the filaments of fabric, because it did not form a crust on the surface. We discovered the presence of a transparent, homogeneous, reddish substance, such as is shown by particles of dried-out, bloody crusts seen by microscope. This substance was in no way crystalline, it filled the interstices of the threads of the smock and formed a varnish around the microscopic filaments of hemp composing the threads of the fabric, a fact which explains the very evident starched condition of the fabric in the area of the stains. This substance was a bit sticky and the thin, angular fragments conserved on their surface the impression of the microscopic filaments from which they were separated.

But in contrast to that which happens: 1) for residues of brown water of dried-out dung; 2) for fragments of bloody crust taken from stains actually formed by blood, the substance of the stains submitted for our examination did not soften at all in water nor in solutions of sodium sulfate or sodium phosphate. Hot and cold acetic and hydrochloric acid equally left them completely intact. Ammonia and solution of potassium blanching and softened the fragments of the substance of the stains, but without dissolving them. It was the same for ether and carbon disulfide, the action of which, however, was less pronounced. Hot concentrated sulfuric acid dissolved all the fragments of the substance of the stains quite rapidly, as it does for most varnishes and resins, at the same time swelling and softening the microscopic filaments of hemp without dissolving them.

*In summary:* this resistance to the action of water, acetic acid, hydrochloric acid, with complete solubility in hot sulfuric acid, and incomplete or insolubility in alcohol, ammonia, potassium and ether, proves that the substance of which the stains are composed is not blood, even though it formed on the fabric of the smock stains possessing all the physical and superficial characteristics of blood stains. These characteristics, the only ones which the small amount of substance permitted us to confirm, were, on the contrary, those which belong to material of resins, of dried-out lacquer and other analogous substances, of an origin different from substances of the human body.

The stains were not, then, blood stains. Nor had they any of the characteristics of solubility and composition of residues of water from dung.

They were formed by a substance analogous to that of resins and of lacquer which had congealed and would have dried out after having saturated the fabric of the smock.

By reason of their chemical nature and their resistance to external and chemical agents, they could not have been recent and could have resisted two or three complete washings of the smock.

In another case submitted for our forensic examination, the stains present on an iron axe, an alleged instrument of crime, had been considered in the investigation as probably formed by blood. They were numerous, reddish, without an ocre taint, and of width of about 1-6 mm. Some were circular, with a very finely jagged periphery; most were irregular. Also were found some of the same taint, of a poorly deter-

mined periphery. All were very thin, not projecting above the surface of the iron. The jagged border of those presenting this characteristic were the only parts projecting slightly. All were not very shiny or of a dull tone, except the very fine tracing slightly projecting at the periphery of some, which was shiny, and of a crystalline appearance.

This dull appearance of the stains became even more evident when they were examined at night by the light of a lamp. Here, instead of reflecting the light, showing a taint of shiny, brown-red, they stayed a duller tone than that of the polished iron bearing them. Their surface by magnifying glass and by the naked eye was delicately rough. Subjected to the action of water, they did not change their appearance; hydrochloric acid dissolved them, returning to the iron its brilliance. The powder obtained in scraping them, and submitted to microscopical examination after the appropriate procedures, showed no trace of red or white blood cells, nor of fibrin. But it allowed us to see small, irregular, angular fragments, similar to those described by M. Lesueur<sup>1</sup> and by one of us, which showed the reactions specific for iron rust.

**Note on blood stains mixed with epidermis and lanugo of a new born.** The microscope shows in the blood stains the very anatomic elements which themselves constitute blood, and thus permits determination of their nature with more precision than methods based on the simple phenomena of coagulation and coloration; but it permits in addition recognition of the nature of foreign bodies, other than blood, which can be mixed in with the material of the stains and can sometimes furnish previous medico-legal indications, in the circumstance where the nature of these bodies oppose the findings uncovered by chemical reagents.

The following case, where we had been called upon with M. Lesueur to determine whether a newborn had been enveloped in a skirt, is a striking example, supporting the preceding remarks, which apply to cases whose number is more than likely to increase greatly.

On the skirt sent to us, we noted that, of the stains it bore, some are reddish, more or less pale, like stains formed by mucus or bloodied serous liquid; these are of the greater number. Most of them starched the material a bit. The other stains, smaller, are of a deep brownish red, like stains a bit old and formed from pure blood. Many of them are superimposed on the preceding stains and stand out by their deeper color, besides, some of them strongly starched the cloth and some of them even form a crust. It is not difficult to recognize, particularly for people accustomed to practicing childbirth, that many of these stains lie on larger stains, very pale, of a diffuse periphery, slightly yellowish, like stains, formed from urine or water from the amnion. They emit, besides, an odor of urine, a certain flat odor, like a mixture of urine and the waters of childbirth, a very pronounced odor, which is accentuated on leaving the skirt rolled up for twenty-four to forty-eight hours in a slightly humid place. Some of the bloodied stains, superimposed on the odoriferous urine-like stains, had their edges blended

into these stains as if the blood had touched the cloth while it was wet and had immediately mixed with the moisture of the latter.

The back part of the skirt bears bloody and urinary stains of the same appearance as the preceding; but they are larger and their edges are more blended together. These stains are situated toward the middle of the upper part of the skirt, on either side of the vertical seam which runs down it, and reminding one by their situation and general disposition of those which the buttocks, wetted by a bloodied liquid, would produce if a person were seated or lying down. Still on this side, but lower, nearer the hem of the skirt are four large irregular stains, of a width of 4-12 cm; one to the left of the seam, three remaining to the right. They are reddish, like stains formed by blood running on a wet cloth, and their edges appear as if washed, blended into the large, slightly yellow stains with a strongly urinary odor, by which almost all of this part of the skirt is impregnated. They also strikingly starch the cloth. All these stains traverse the fabric, but are more marked on the side of the skirt turned toward the body than on the opposing side.

The outside of the skirt is soiled by tracks and stains of grey mud, evidently coming from rubbing against mud or earth or dust while still wet.

After having cut out appropriate strips, taken from the principal stains, we dipped them into as many watch glasses containing sodium sulfate with the addition of a few drops of glycerin. The substance of the stains, once softened and gradually swollen by saturation without being dissolved, were removed with care by scraping, and submitted to examination by microscope.

The material of the deepest, thickest stains forming a crust showed *red blood cells*, some intact, biconcave, circular, others a bit swollen, becoming almost spherical and a bit jagged, as they become in blood exposed to air; but they were still immediately recognizable.

These stains showed in addition a rather considerable amount of fibrin, which the action of pure water permitted us to isolate and discolor by washing out the red blood cells in such a way as to render their fibrillary aspect clearly evident. In the fibrin were some white blood cells, small in number, clearly recognizable. Now, it is known that fibrin does not form fibrillary clots in menstrual blood, which flows normally and mixes with mucus of the matrix, and that fibrin is not found in stains produced by menstrual blood on cloth. Besides, we did not find in the material of any of the pale or dark stains which we examined, white corpuscles called mucus corpuscles which accompany menstrual blood in great number, and which are easily found in the stains in a proportionately greater amount as the stains are paler, have more mucus and are less bloody.

The reddish stains, paler than the preceding, not forming a crust on the cloth, which however they starched a bit, showed red blood cells like the preceding stains; but they enclosed neither fibrin nor white blood cells.

The material of the stains additionally demonstrated ele-

ments which, even though coming from the surface of the human body and its membranes, are foreign to blood. These are *epithelial cells*, polygonal, finely granulated, with an oval nucleus, isolated for the most part, some, however, united in sheaths as a result of their imbrication. These cells are similar to those of the vagina and external genitals of women. They could have been carried out during childbirth, and deposited on the skirt either by the blood which flows in such a case, or during the passage of the infant. They are, it is true, similar to those one finds in menstrual blood; but they were not accompanied, on the stains of the skirt, by the mucus corpuscles which always accompany menstrual blood in great numbers.

In the material of these stains, were found other epithelial cells, cuboidal, thin, transparent, of pale edges, non-granulated, some folded, others marked by fine, irregular lines. The greater part were united in epidermal strips or laminae of a width of 0.1-0.5 mm. These strips or laminae were larger and more numerous than those which naturally detach from the human body and adhere to the parts of clothing immediately contiguous to the body. In the thickest of these strips, formed by many rows of superimposed cells, the most pronounced were smaller than the others and provided with a nucleus. These are characteristics belonging to the still thin *epidermis of the foetus* when removed by fairly rough scrubbing or scraping, and which one does not find on epidermal sheaths naturally detached from the surface of the human body; these latter, indeed, never show nucleated cells.

Along with these small, epidermal sheaths, whose structure leads one to consider as coming from the surface of the body of a foetus, were found a few free *lanugo hairs*, detached from their follicles, small in number, but easily recognizable. These hairs had the characteristics of those found on the surface of the foetal body during childbirth. They were pale, uncolored, faintly striated lengthwise, without coloring material in their thickness, a width of only three-hundredths of a millimeter, without a medullary canal, of pointed end, a bit irregular, with a small tapered root. These characteristics, as one knows, are in no way those of pappus of the adult human body, the diameter of which varies from 6 to 8 hundredths of a millimeter, the free end of which is a bit flat, the substance provided with coloring matter, and the center hollowed out by a medullary canal, interrupted or continuous, and filled with a granulous marrow more or less opaque.

In addition, we have found in the material of these stains, as in all the others of which we'll be speaking, a few *grains of starch* and some irregular grains, of variable volume, which the appropriate reactants have shown us to be of a mineral nature. Of these diverse corpuscles, found in almost all dust originating outside the human body, we limit ourselves to pointing out their existence, without pursuing the matter further; for no conclusion whatever can be based on their presence, any more than on their absence.

We had to study an oval, irregular stain, 12 mm wide,

found on the large hem on the bottom of the skirt. It was greenish brown, becoming greener when scraped. These external characteristics being those found on stains formed by meconium, we studied it according to tried procedures for the examination of the latter. We found there polyhedric cells, filled with a greenish-brown, granular material. These cells had all the characteristics of those of plant parenchyma; some isolated, others still united in variable number; bundles of punctured vessels and trachea of herbaceous plants, such as one finds in sorrel, spinach, or the parenchyma of various crushed and ground or cooked leaves. This stain enclosed no elements of meconium at all, and its color derived from grains of chlorophyll or green coloring matter of plants, noted in the cells of which we just spoke, and having, by dessication, lost in part the vivacity of their green color.

*In summary:* from the preceding examination it results that: 1) the stains of this skirt contain, besides blood, elements which could have been mixed in only by immediate contact with the sexual parts of woman; 2) the disposition of these stains in back, in the area of the buttocks, the presence of large stains also in the back part, much lower than the genital organs, demonstrate that all, or almost all, these stains come from blood which flowed from the genital parts of a person giving birth when they were formed; 3) the presence in large blots of these pale stains, of a very pronounced flat odor, similar to that of a mixture of urine and amniotic liquid, together with the presence in the material of these stains of sheaths of epidermis similar to that of a foetus, and especially of lanugo hairs of a foetus, show that these stains come from the blood of childbirth, and that the foetus must have been in contact for a more or less long time with, or even enveloped in, the skirt which bears them.

## Mucus Stains

**Examination of a stain allegedly of the nature of meconium and formed by the material of bronchial and pharyngeal expectorations.** In the assessment mentioned in the preceding section, with which we had been charged, along with M. Lesueur, a bed sheet, on which the childbirth allegedly took place, showed a stain which its appearance and various circumstances mentioned in the investigation led us to consider as being formed by meconium. After having observed the external characteristics of this stain, we conducted our examination of it following the procedure we outlined in a preceding work.<sup>2</sup>

Twelve centimeters from the edge of the sheet was an irregularly circular stain 2 centimeters wide, a pale greenish yellow. The portion of fabric bearing it was cut out, and one end of the cloth, upon being plunged in water, notably softened and swelled when the liquid reached the stain by imbibition. Examination by microscope showed it was composed: 1) of a transparent, homogeneous mucus, striated like that of viscous expectoration, produced in the case of chronic laryngitis and in expectorations called "*hem*"; it held in suspension only a small number of molecular granulations;

2) in addition this mucus held in suspension cuboidal epithelial cells, similar to those of the pharynx and mouth, but small in number; 3) other spherical epithelial cells, 2-3 hundredths of a millimeter wide, of which some were very granulated and showed their central nucleus only after the action of acetic acid which blanched or dissolved the granulations. These cells are always found more or less abundantly in expectoration coming from the bronchi, larynx and the back of the throat; 4) this mucus contained especially a large number of leukocytes called mucus corpuscles, irregularly spherical, one hundredth of a millimeter wide. They were accumulated either in irregular masses, or in lines more or less long, parallel to the mucus striations. Their characteristic nuclei, at first invisible, showed up most evidently on contact with acetic acid. This acid rendered the mucus more clearly striated, and gave it a fibroid appearance more pronounced than that which it had beforehand. This reagent has the property of modifying mucus, permitting a distinction between semi-solid and solid mucus and coagulated fibrin; for it swells fibrin, making it lose its striated aspect and its fibrillary disposition.

434 These characteristics being those one finds in products of pharyngo-bronchial expectoration, and not in meconium and other mucus materials, we concluded that this stain was produced by expectoration which had accidentally fallen on the edge of the sheet.

### Seminal Stains

**Note on the distinctive characteristics of seminal stains and stains of fecal matter.** Stains of a seminal nature were found on the shirt of a young girl, less than eleven years old; which were accompanied by stains of another appearance, which, at first look, had been considered as *natural and physiological exudations of female genital organs*. Starting with this idea, the first expert concluded that these stains were all located on the tail of the front of the shirt.

We were judicially committed, by request of the first expert, to verify the nature of these stains. On one of the tails of the shirt, near the edge, we found two greyish, pale, irregular spots, slightly starched, penetrating the fabric by absorption, but more marked on the side turned toward the body than on the outside. One was 5 millimeters wide, the other narrow, with jagged edges, of the same width and 32 millimeters long. On the opposite tail of the shirt was a large, irregularly semicircular stain, with the edge of the shirt as base, 13 centimeters wide, and 11 centimeters long. All around it for a distance of about 10 centimeters, but mainly above, were many small stains varying in width from a few millimeters to 2 centimeters. Their form was not very regular and their periphery sinuous, or jagged, in places. Those nearest to the large stain merged with it in places, rendering the periphery irregular. These stains were pale, greyish, slightly darker at the edge than toward the center without a yellowish taint. They were more easily perceived at night by  
435 lamplight than by day, because they compared more

strongly with the tone of the fabric, without shining, however. This characteristic is found in diverse stains produced by mucus liquids of the human system. They traversed the fabric, being, however, a bit more marked on the side turned toward the body than on the outside of the shirt, even though these were whiter and less dirty than the other. The disposition of the hem, in addition to the manner in which the shirt was stained, enhanced the distinction between the inside and outside of the shirt.

All these stains slightly starched the fabric, the smaller more so than the larger; even though the starching was evident, the folds of the cloth, once formed, were conserved more in the area of the stains than in non-stained areas. The large stain had in addition to its greyish tint a sullied shade, which the small ones did not have, and which rendered the larger one darker.

On the large stain, a bit to the side, on the side of the tail of the shirt turned toward the body, were irregular stains or mackles, brown or greenish-gray, dull, like mackles of excrement; they were thin, without forming, or almost without forming, a crust. There were, principally, three of them, 8, 15 and 23 millimeters long, of a lesser width, and variable in their different points of extension; they were united by irregular streaks of the same appearance, seemingly formed by rubbing the substance of the principal stains while they were still fresh. Their edges were jagged, like those of impressions left on folded cloth by a colored substance.

After having noted the various external dispositions of the stains, we cut out a certain number which we then separated into two halves, with the purpose of submitting one to microscopic examination, and the other to the action of chemical reagents.

**Examination by microscope of the substance of the pale stains, presumed to be of seminal nature.** We cut out in thin  
436/ thongs the portions of cloth bearing each of the stains whose nature we wanted to determine by microscope. We then dipped them by one of their ends into as many watch glasses containing a little distilled water. At the end of an hour or two, the water having slowly wet the cloth by absorption and capillarity, the stains were swollen and projected a bit from the surface of the fabric, a fact which required much attention to be seen.

At the same time, they became shinier than they had been, and the pale, gray stains, suspected to be of seminal nature, took on a bit of a mucous or gelatinous appearance.

We then scraped each stain with a clean bistoury to remove the substance covering the fabric, and that which penetrated between the threads by absorption. We then submitted the matter thus removed, separately for each stain, to microscopical examination. The microscope showed us in the substance obtained, as it was just pointed out, a certain number of filaments presenting all the characteristics of those which compose threads of hemp. Between them we easily perceived a great quantity of homogeneous, scarcely grey, transparent substance, such as one finds in semen and

other mucous substances of animal origin. They showed up either in flakes of sinuous contour, such as we have represented in the design attached at the end of this report, or in more extended layers interposed between intertwining filaments. We saw at the same time a large number of very small, threadlike, pale, greyish bodies, 5 hundredths of a millimeter long, one thousandth of a millimeter wide, ending in a swelling or head of darker contour, ovoid in the form of a flattened pear, 5 thousandths of a millimeter long. These characteristics are those of spermatozoa, elements characteristic of semen, and one finds them in no other substance coming from the human body.

/437

They were as numerous and as concentrated as in seminal fluid, such as it is when just ejected by ejaculation. They were whole, flexible, and there was only a very small number broken by the maneuvers of preparation. Having added to this a drop of dilute acetic acid, we saw the mucous substances in which were suspended the elements characteristic of semen, dissolve and these spermatozoa stayed intact and were more clearly perceptible than before.

We found, in addition, in the material of these stains placed under the microscope, some rare, prismatic, elongated crystals terminating in a point, reminiscent of magnesium phosphate crystals. As is known, these crystals are often deposited in semen during its cooling after having been ejected by ejaculation. We also noticed some mucus corpuscles and some polygonal, flat cells, provided with nuclei, as are epithelial cells of the urethral canal, elements often carried out in small number during ejaculation.

All these elements being found in semen, and accompanying here microscopic filaments called *spermatozoa* which essentially characterize sperm; these existing in as great a number as on the fertilizing liquid, and furnished exclusively by the genital system of a man having reached the age of puberty, we concluded that:

The gray, pale stains submitted for our examination as possibly being of a seminal nature, are indeed composed of elements characteristic of semen, such as one finds in semen cooled or dried out after ejaculation.

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We also found mixed with these elements: 1) some microscopic grains, irregular, dark, such as one finds in most dusts originating outside of the human body; 2) some rare starch granules, such as one finds on the surface of many whitened fabrics and in many dusts; 3) polygonal, thin, folded, transparent cells without nuclei, almost without granulation, similar to those which incessantly detach from the epidermal surface of the human body, and most of which remain adherent to clothing applied directly to the skin.

**Examination by microscope of irregular brownish stains accompanying the preceding, but which offered the superficial characteristics of stains of fecal matter.** Under the microscope the material of these stains showed the elements found in the pale stains described previously as elements of semen. But we encountered a considerably greater amount of the following microscopic particles:

1) Greenish granules, irregular with blunted angles or sinuous contour, such as are contained in bile and fecal matter with which biliary liquid is mixed.

2) Cells and tracheae of vegetable matter, such as most vegetable substances contain which serve as food for man, and which stay in the fecal matter in whose constitution they participate.

3) Yellowish globules and droplets, strongly refracting light, ether-soluble and offering all the characteristics of fat globules, and drops which one finds in a certain amount in fecal matter.

4) Finally, we found a certain number of microscopic bodies, ovoid, about 7 hundredths of a millimeter long, provided with a transparent homogeneous wall, rather thick, the external contour a bit embossed and a regular cavity filled with a granular, greyish substance. These bodies offered all the essential characteristics of eggs of the intestinal worm *ascarides lumbricoides*, which one finds in fecal matter of individuals affected by the presence of these intestinal worms.

By this examination, we were led to conclude that these irregular, grayish stains, considered in the report of the preceding expert as coming from a natural exudate of the genital system of woman, do not contain elements characteristic of these materials, all recognizable by microscope; that they contain the principal elements specific to stains formed by fecal matter; that the stains were actually formed by fecal matter, coming from the anus, cleaned by the shirt after defecation; that the elements of these stains were mixed with those of semen, either because the seminal fluid was ejected onto them, or, on the contrary, that that part of the shirt stained by the semen was stained by the fecal matter.

In addition: 1) the less wear on the tail of the shirt bearing the stains of excrement, compared to the opposite tail, which bore no similar stains; 2) the nature of these stains, actually formed by elements of fecal matter, and not by mucus material coming from the genital system of woman, has led us to conclude that:

The tail of the shirt bearing these stains, as well as the stains of a seminal nature, is not at all the tail of the front of the shirt, but the posterior tail, contrary to indications of the report, following the allegation that these dark gray stains were of a mucus nature.

**Study of the chemical reactions presented by the stains submitted to examination by experts.** Even though the characteristics previously described left no doubt as to the nature of the stains we had been studying, since we had found the very elements which compose seminal fluid in the human body, we submitted them to the chemical reactions indicated as serving to distinguish different sorts of the suspected stains one from the other; for this we used the portions of stained fabric which we had put aside for this purpose. (See Lassaigne. *observations sur quelques réactions que présentent les taches spermatiques avec les taches albumineuses et autres taches analogues. Annales d'hygiène et de médecine*

*légale*, Paris, 1858, in - 8°, t. X, p. 405).

The heat of incandescent charcoal acting far enough away so as not to redden the fabric instead of producing a dark nankeen yellow on the stains, as happens when one operates on a white cloth stained with semen, produced a hardly-visible nankeen yellow tint. This might be attributable to the dull tint of the whole tail of the shirt worn for a long time, which showed the stains submitted for our examination.

*Cupro-potassium sub-tartrate* which, applied to seminal stains on white linen, colors them a greyish-blue, colored what we were studying a violet a bit pale and highly visible, as it colors stains of albumin; this might be attributable to the fact that, these stains being placed near the stains of fecal matter, the fecal liquid portion, which is mucus and albuminous, inevitably infiltrated the fabric; in mixing with the seminal liquid, it modified and masked the chemical reactions without, however, changing in any way the characteristic elements we have described.

Nitric acid at 40° changed the seminal stains, located farthest from the fecal matter, to a pale yellow; this color, at first not very visible because of the pale tint of the fabric, became more pronounced under the influence of heat. On the contrary, this acid changed to a yellow veering toward orange, stains of the same appearance as the preceding, which the microscope demonstrated to us as being of a seminal nature, but which located near the stains of fecal matter, must have imbibed their mucus and albuminous liquid portion.

In summary, the use of chemical reagents furnished us with no new proof concerning the nature of the stains of which the microscope had directly shown us the constitutive anatomic elements.

This is due to the mixture coming from the infiltration into the fabric of the liquid part of neighboring stains, of which the microscope demonstrated the superimposition in places, showing the elements of semen mixed with those of fecal matter in certain places of the stained fabric.

This mixture, which is not rare in cases of an assessment of this type, takes away much of the value of characteristics derived from coloration which the stains show on contact with certain reagents. These characteristics, to which a few authors still attach a certain importance, have, however, no importance in the presence of *spermatozoa*. Their presence is so exclusively characteristic of seminal fluid, that one can claim any stain containing them as being of seminal origin,

and one cannot affirm their seminal nature until the existence of spermatozoa has been confirmed.

Moreover, characteristics derived from coloration can only be confirmed if the stains are found on white linen; one cannot produce this coloration when the stains are found on colored fabric; this is what happened recently to one of us (M. Robin) during an assessment in which the only stain to be examined was found on grey wool pants; then, in proceeding as mentioned above, the spermatozoa and other elements of semen were discovered as clearly as in fresh seminal fluid.

*Conclusions.* The results of our examination have led us to conclude: 442/

That the pale, gray stains, by which the shirt is covered in several places, are of a seminal nature and offer all the characteristics and disposition of stains coming from seminal fluid ejaculation.

That the dark brownish stains, smaller and less numerous than the preceding, and mixed with them, especially with the largest, are constituted by fecal matter, such as would voluntarily or involuntarily escape from the anus of a child.

That the nature of these stains, their situation on the inside of the tail of the shirt which is the dirtier and the less used, show that they are found on the tail of the back of the shirt.

It follows that the seminal stains mixed with them are also on the side turned toward the body of the tail, of the back of the shirt and not on the tail of the front of this shirt.

That these stains, which are of a seminal nature, could have been produced on the tail of the back of the shirt by an ejaculation brought on by the rubbing of the erect penis between the two thighs of a child clothed in this shirt, of which only the tail in front would be lifted.

That the three small seminal stains, which the tail of the front of the shirt showed, could have been produced by semen which remained on the thighs of the child, or by contact with the tail of the back, which was the part principally stained.

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## On Semen and Seminal Stains in Legal Medicine\*

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417 Determination of the nature of stains has taken on a more considerable importance at the Palace these last years, perfectly justified by the progress realized in this process by chemistry and histology, as well as by the unexpected light it has thrown on famous trials. There is no great risk of being contradicted in affirming that every time a preliminary inquiry must grapple with an intelligent criminal, who appears to have left no trace of his passage, it concentrates its foremost efforts on the investigation of stains and imprint evidence. And this effort is quite often crowned with brilliant success.

By such study, the innocence of the accused can be promptly demonstrated; in other cases, the culpability has been rendered evident, circumstances of the crime established or the scene of the murder reconstructed in a more precise and clear fashion than with a witness, often an unknowing child or an unreliable individual. Analysis of stains today is also among the standard procedures, and not the least important, of forensic physicians.

At Lyon, Professor Lacassagne was to annex a perfectly equipped laboratory to his amphitheater, devoted exclusively to this type of research; elsewhere, in lab courses in legal medicine, the students practice special manipulations.

At Paris, Brouardel, Vibert and Pouchet gave great impetus to this research and left us studies of the highest interest on these questions.

418 Blood stains were studied especially, and reasonably so, for if the crude material proof of their nature has today become a sort of game, it is not the same for their *interpretation*, which demands a great prudence and very special wisdom on the part of the expert, so great is the danger in having them say more than they can. Blood abounds, it runs in the streets, a scratch makes it flow and a number of professions cannot be practiced without its blemish. It is not the same for seminal stains, whose presence in frequent cases has an absolutely precise significance and constitutes an irrefutable accusing witness. Their interpretation is extremely easy, for, rather often, the role of the expert consists in saying yes or no as to the presence of spermatozoa. Their identification has been generally considered as very simple,

certain exceptions reserved, of course, and I have always reckoned, for my part, that with the precepts decreed on this matter by Roussin, Robin, Lacassagne, Renaut, Brouardel, Pouchet, Gérard and many others (1), in arming oneself, in addition, with a lot of patience and an appropriate microscope, one could always do a creditable job in these affairs. I have in any case considered any addition to these precepts as perfectly superfluous.

I have sometimes had, it is true, considerable difficulties in characterizing certain stains. About ten years ago, Coutagne and myself spent three weeks in isolating a few complete spermatozoa in a case of rape of a four-year-old child. More recently, Lacassagne had a few equally extremely difficult assessments. Notably, one concerned stains dating from a month before, which had been washed by the mother of the little victim herself. It is easy to see that it was impossible to succeed, even though the place occupied by the stain was still visible; the able expert succeeded quite well in isolating some filaments which could have been tails, corpuscles which could have perhaps been heads more or less altered by the washing, but a professional opinion cannot be expressed based on such observations. If, instead of semen, Lacassagne had been working with blood, he would have needed an hour at most to affirm, by the preparation of hemin crystals, that the stain was really made of blood, in spite of the washing, even if it had been more effective. With the reaction of guaiacum, only a few seconds would have been sufficient, if it were negative, to be certain that it contained no trace of blood! After having passed long hours and sometimes weeks in examination of a seminal stain thread by thread, one often arrives at that semi-certainty, as embarrassing for the expert as it is useless to the judge.

These reflections caused Lacassagne to wonder if it weren't possible to find a simple and rapid reaction for sperm stains, equivalent to that of guaiacum, and secondly, to determine exactly if, from new notions recently acquired about the anatomy of spermatozoa, sufficient certainty for expressing an opinion could not be attained by examination of a tail or a head as well as of a *whole spermatozoan*, which up to now has everywhere, without exception, been considered indispensable. The results of the research I undertook in regard to this question will now be presented.

### The Study of Semen

Semen (*σπέρμα*; seed) is a thick, viscous liquid, a bit flowing, of an odor *sui generis* called "spermatic", and which

\* Translation of: "Du Sperme et des Taches de Sperme en Médecine Légale." in *Archives d'Anthropologie Criminelle de Criminologie et de Psychologie Normale et Pathologique* 10: 417-434 (1895), continued in the next volume.

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has been compared to flowers of a chestnut tree, shredded hoof, sawn ivory, flour dough, gluten, etc. Its consistency is variable: thick, almost a gelatin, in a vigorous man after a long abstinence, it becomes very fluid, scarcely milky, in those who abuse venereal pleasures. In the first case, it is an opaque white, bordering on yellow or grey, almost pearly, clotted, nonhomogeneous, because striations in an uncolored, transparent liquid, appearing dark by a phenomenon of refraction, engulf the masses of gelatin. In the second case, it is rather homogeneous, more or less milky, a little translucent. In a degree of even more considerable impoverishment again, after several repeated acts of coitus in a short period of time, it is transparent and shows only a few small whitish points or striations; at the same time the spermatic odor becomes weaker and weaker and even null. Without knowing the reason, semen can be a pure pearly white, or bordering a bit on yellow which is the most usual hue, or a dull greyish tint, or even, but more rarely, frankly roseate, in the same individual. This last hue is, it is true, sometimes due to blood, but is the exception outside of old age; according to Robin, this coloring substance is formed by vesicles observed by him. Coutagne and myself have had to examine stains of rose-colored semen, and the accused assured me that his semen was always of this color; his interest, however, lay in maintaining the contrary.

Semen is heavier than water: its density can attain 1.037 and oscillates between this number and 1.027 in a healthy man; it is mucilaginous rather than viscous, weakly alkaline. Exposed to air, the thickest semen gradually loses its gelatin-like consistency, becomes fluid and separates into two layers, one upper, transparent and uncolored, the other creamy and white. It is thus that I have seen it in samples I have received from various origins, but certain authors insist that, on the contrary, it becomes thicker. Acetic acid and heat do not coagulate the clear supernatant liquid, but if hot acetic acid is added, the liquid becomes intensely cloudy; likewise, if potassium ferrocyanide is added. I will return to this.

Alcohol immediately gives a white precipitate, milky at first, then curdled. The precipitate, examined by microscope, is very finely granulated, engulfs spermatozoa and sometimes large crystals.

In drying, on glass for example, semen leaves a translucent mass of a fatty, granulated, yellowish appearance; if water is added, the primitive semen is not changed, as has been claimed, due to most of colloidal substances having become insoluble. There remains an abundant, clotted, dull grey, unflowing residue, not more than the supernatant liquid which is scarcely cloudy after being left alone. It is also said that the odor of semen returns under these conditions; I have never confirmed it, even with the help of low heat. Moreover, there is a lot to say about this odor, which I have never found in any of the various animal semens which I could appropriate. It is said that addition of blood to dry semen regenerates the primitive odor, and it is affirmed that it is due to spermine itself, as will be explained further on. Semen of sperma-

torrhea and of sudden death has no odor.

According to certain authors, its flavor is peculiar, very bitter; according to others, simply a bit salty or flat. It is the last which appear to be true, but it is possible there are variations. Semen, in effect, is a very complex liquid, formed from products of secretions of several glands, each secretion having its very distinct characteristics which the limits assigned to this work do not permit me to present in detail. They are:

1) *Testicular secretion*, formed almost exclusively by spermatozoa, thick in bulk, or pasty, and semi-liquid, sometimes creamy, dull white, opaque, or bordering a little on yellow, non viscous, *without odor*. According to Robin many spermatoblasts are found.

2) Liquid of the *sinus of the vas deferens*, brownish or yellowish grey, with various granulations and numerous epithelial, prismatic cells.

3) The liquid of *seminal vesicles*, *dense, without odor, alkaline*, clotted or creamy, unflowing, sometimes gelatinous, yellowish grey, semi-transparent, not opaline. In the elderly it is reddish brown, and sometimes contains red blood cells. It appears that this is the first liquid ejaculated in infancy and the last in old age. After repeated coitus it forms almost the totality of semen. It contains polyhedric epithelial cells, leukocytes, *drops of an oily appearance*, coloring strongly with reagents and especially with crocein which permits recognition of the bizarre forms which the drops under the top slide take on, stretching out in every direction. Finally, curious round or cylindrical concretions of a diameter varying from 1/100 of a millimeter to one or even two millimeters, according to Robin who has described them at length, and called "*sympexions*". They are uncolored except in the elderly where they are sometimes roseate, rather often branchy or areolate.

These granulations, noted in all the treatises of legal medicine and which can perhaps be confused with the amyloid granulations of certain German authors, have never been used, that I know of, in the diagnosis of stains, and I will not discuss them further. However, I will recall that the most voluminous often engulf spermatozoa, which can be easily isolated with acetic acid.

4) *Prostatic humor*, or "prostatine of Blainville", is a fluid liquid, neither viscous nor flowing, milky, opaline, cloudy giving a weakly alkaline reaction. According to Robin, it has no odor, but Fürbringer, who recently performed a good study on fifty-one cadavers and twenty-one living adults, constantly found the specific odor of ejaculated semen. It is composed of an uncolored, clear liquid holding very fine granulations in suspension, amyloid according to Fürbringer, fatty droplets, flat and cylindrical epithelial cells, grouped in strips, hyaline balls due perhaps to colloidal degeneration of *epithelium*; finally according to Fürbringer, some constant and characteristic elements, uncolored, refringent, round or oval, rarely angular, to which is due the cloudy appearance of the solution; the largest have the diameter of a red blood cell, whereas there are some exces-

sively small endowed with Brownian movement. According to Robin, it is to the product of the prostate that semen owes its appearance, and though he asserts, in contrast to Fürbringer, that it has no odor, he admits that it is, by its mixture with other humors also without odor, that spermatic odor develops. This is why it is lacking after repeated coitus and the sperm becomes greyish, not very opaline and clear.

5) Humor of the *prostatic utricle* ("male uterus" of some others) is insignificant in relation to what interests us and contributes only very little quantity to the formation of semen.

6) That of the glands of Méry (Cowper's gland) is more interesting, for it is often so abundant that, during erection, it wells up in a liquid, limpid-like crystal, viscous, stretched out in threads, salty, alkaline, without odor. This humor often forms starched stain, in drops, but difficult to determine, for up to now, the specific principle has not been found, and besides it contains absolutely no morphologic element. I except from this however a few uncharacteristic epithelial cells.

#### Histological elements of sperm

##### 1) Spermatozoa (σπέρμα and ζῶον)

(*Animalculi e semine, vermiculi minutissimi* (Leeuwenhoek, 1677) *Filamenti spermatici—Vers spermaticques* (Spallanzani); *Spermatic Animals* (Procope, 1755; Needham, 1750 and Spallanzani); *Minutae bestiolae* (Halter, 1765); *Spermatozoa* (Duvernoy, 1841); *Spermatic Filaments, Seminal Filaments* (Henle, Koelliker); *Zoosperm* (various authors); *Spermozoaires* (Bory de Saint Vincent); *Zooblastes, nematospermes, nemospermes* (Bory de Saint Vincent); *spermatozoaires, entozoaire of sperm or spermatoxies* (Baër); *Treniadosa pseudopolygastrica* (Ehrenberg); *Macrocerus, of the Cercozoa family* (Hilt); *Cephaloides* (Czermack); *Microscopic cercaria or cercaria of semen* (Cloquet, 1827).

*Samenkorperchen* (seminal corpuscles) (Schweiger-Seidel); *Samenthierchen* (Koelliker, 1841) *Spermatozoid, Spermatozoon, Spermatozoa, Spermatic particles* (English).

Without wanting to repeat here the well-known history of this question, I will recall however, that it is in a letter dated November, 1677 and entitled *Observations on the small animals of human semen* that Leeuwenhoek first made known the spermatozoa. Ham<sup>2</sup>, supposed to have been one of his students, had observed them living in the semen of the nocturnal pollution of a patient with gonorrhoea, and hastened to share his discovery with the illustrious professor. He told him that he had already seen them, but dead, after injection of turpentine into the patient. Leeuwenhoek looked for them again and found them in the semen of a great number of vertebrate or invertebrate animals. He compared them to tadpoles of frog, and believed that in man and dog there are two types, perhaps of different sex<sup>3</sup>.

He confirmed that they come exclusively from testicles, and an important point, *that they don't come from putre-*

*faction*, as many other small animals, infusoria, for example, a point of great interest then. Nonetheless, for many years they were only considered as foreign animalculi and this is, I suppose, the reason why it is only in recent years that the presence of spermatozoa was considered as the essential characteristic of seminal stains, and acquired a legal value. Procope, in a little book, which moreover, he did not sign (*The Art of Making Little Boys*), pleads the question rather spiritually, and believed that he had proven that spermatozoa are only the accessories, the accidents, so to speak, in semen. He says that Hartsoeker (1678?) a contemporary of Leeuwenhoek, had remarked that semen obtained after several ejaculations contained no more spermatozoa; the semen was, however, not less fertile; as proof, the numerous disappointments incurred by those who speculated on Hartsoeker's discovery. . . . For Buffon, too, spermatozoa and infusoria were of the same origin, or almost, for he often seems to confuse them. Despite the works of Spallanzani, it was in reality Prevost and Dumas (1824) who definitively demonstrated that it is not the odor of semen—the *aura seminalis*—which is the fecundating principle but the spermatozoan. A simple reading of names given to spermatozoa by different authors—names which we reproduced above—is as convincing as a long history as to the ideas they had.

Spermatozoa are filamentous anatomic elements found exclusively in semen; they are uncolored, hyaline, inflated in man and most higher animals in one of their extremities, commonly called the *head*, tapering into a long, extremely tenuous cilium, endowed with its own movements, called *tail* or *flagellum*. They are quite rightly compared in form, and also in movement, to tadpoles of the frog, but their tail is proportionately much longer and finer than that of tadpoles.

The relative and absolute proportions of spermatozoa of man are very fixed, more fixed, in any case, than those of any other anatomic element, blood cells or pus, for example. Also, contrary to what has been professed up to now, the fragment of spermatozoa most easily found in old stains, the head, can by itself, in my opinion, if studied well, and rigorously measured, give absolute certainty as to the presence of sperm.

In relation to the technique of assessment, I will note the influence certain coloring reagents have on these dimensions and I will specify more rigorously the different dimensions obtained. The total length of the spermatozoon of man, more difficult to rigorously measure due to the extreme tenuousness of the end of its tail, seems the only part to vary appreciably: it is between 0.048 mm and 0.058 mm. Dujardin notes that the caudal filament represents quite exactly 1/10 of the total length, say 0.050 mm, whereas that of the head is 0.005 mm. As an average of numerous series of measurements, designed exclusively to find out the ratio of head to tail, I found the head having four divisions of the micrometer, the length of the tail varying between 37 and 45, with the great majority being between 40 and 41. The length of the head is of extraordinary fixity, when it has not been deformed by accident, and I do not believe there are

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variations greater than 0.0003 mm, more or less, which is within the limits of precision of our instruments, for the markings of ocular micrometers are too rough. The width of the head, seen flat, is 0.0035 mm; its thickness cannot be determined; face on, it is pyriform, and towards the point about 0.0015 mm, toward the base about half the width.

The tail measures, toward the head, i.e. the middle segment, 0.001 mm thick, then it *regularly* thins to end in a point so tenuous that is difficult to perceive the end with the best instruments if some type of article is not used.

Spermatozoa are always uncolored, even in colored sperm, strongly, but unequally, refringent, hyaline. They *appear* perfectly homogeneous in the whole length of their tail, a bit granulated in the head, which is transparent when flat, and permits seeing the granulations which it can screen. At first sight, it appears formed by a single gelatinous substance, but it will be seen that different parts absorb coloring reagents differently which proves they don't have the same chemical constitution. I have tried, uselessly up to now, to employ polarized light in the study of their structure, for the identification of fragments in the analysis of stains.

The *head* or disc of the spermatozoon presents itself in varied aspects, which it is of consequence to know well. It is represented in treatises and articles of legal medicine, almost without exception, in the form of a pear, the small extremity directed toward the front. This is wrong, for when extracting spermatozoa from a stain at least as many which present their head *face on*, i.e. with the aspect of *regular oval*, are found. I have often seen beginners fail to recognize them thus, such was the classical figure fixed in their minds. Something to note, when very rare spermatozoa are extracted from a stain, when only one or two are found, I don't know why, but they always present themselves precisely face on. I made this observation a long time ago. The head seen face on is quite regularly oval, and often shows towards the union of the posterior one-third with the anterior two-thirds a swelling corresponding to the transverse line of separation about which I will speak.

When semen, even from a stain, is treated with a solution of crocein<sup>4</sup>, which of all the numerous reagents which I have tried has given me the most satisfaction, the head of spermatozoa is seen cut just about in the middle, or closer to the base, by a transverse line, separating a little-colored, transparent part from a posterior part strongly impregnated. This line is sometimes distinct, as cut with a knife, but in stains it is sometimes shadowy, blurred, as photographers would say. It is not always straight; I have seen it curved in certain cases concave to the rear, such that the anterior clear part covered the rounded posterior part like a sort of skull cap or crescent. At other times it is oblique or irregular, which must be an accident of alteration or deformation. This separation is variable according to species, and can serve to differentiate sperm of various origins. In man, in seminal stains, the appearance resembles exactly that of an acorn of oak enclosed in its cupula (see Fig. 1) [Note: Figure is not reproduced in this translation]. The contents of the dark part is granulated

and with the use of double staining, it is confirmed that the head is formed by an envelope which eosin colors poorly, and a nucleus it colors well, whereas the iodated solution of Roussin and crocein perfectly color this very thin envelope.

The head of the spermatozoon stained with crocein presents a small, brilliant, refringent point, always situated in the clear anterior part (Fig. 1). In moving the tube of the microscope very slowly, one is convinced it is a small *vacuole*, the diameter of which is essentially that of the tail of the spermatozoon; it is sometimes oval, the large axis directed transversely; more rarely there are two, smaller and unequal. This small vacuole was not acknowledged by Ballowitz in the work (*Centralbl. f. physiol.*, 1891) which he recently devoted to the anatomy of the head of the spermatozoon, and I found only one author who said anything about it: Rollin, a fastidious observer.

After having noted that the head can be placed in such a way that the depression in the form of the hollow of a spoon in one of its faces can wrongly be taken for a nucleus, he says, "this so-called nucleus must not be confused with one or several clean, yellowish vacuoles which form more or less early after the cadaveric death of the spermatozoon in the thickness of the disc". This is effectively what I believed, before making the acquaintance of these lines of Rollin, and my friend Vialleton supported me in this idea; but I should have returned to it since I had positively seen this vacuole on living spermatozoa. Little of the rest is of consequence to me; what is of the greatest interest to me is that this vacuole is almost constant in spermatozoa retrieved from stains and stained by crocein.

All these characteristics, combined with the rigorous measurement of the head in length and width, are such in my eyes that I will affirm in all tranquility the presence of semen in a stain by inspection of *one single head*—whereas, I have the conviction that there is the possibility for any number of errors in the idea, accepted as dogma, that a professional opinion can be expressed if one single spermatozoon is complete (that is, a head with a tail), seen as it is recommended to study them, with magnifications that are too weak, where the head is a point and the tail a striation!

There exists no anatomic element which has any resemblance whatever to the head, appropriately examined.

Valentin, Jensen, Furst, Brunn and Ballowitz described a hat covering the head of the spermatozoa. I have only been able to observe it once, and this at a time when I didn't yet know it was a constant organ in the spermatozoon before maturity. I will then say nothing about it. At the point of the head of the spermatozoon of man, there is positively a small, brilliant point and there exists a similar one toward the tail; but they are not very visible, even with crocein. It is otherwise with animal sperm.

What causes the separation of the head into two parts is that the anterior part of one of the faces is hollowed out like a spoon, as can be confirmed when it is presented in a three quarter view. Thus seen, this excavation which can give the illusion of a nucleus by refraction, as already pointed out by

429 Robin, is readily evident; the other side, on the contrary, is a bit bulging. Seen in profile, the head is pyriform, the large extremity directed toward the tail, the point toward the front, thin and rounded, sometimes inclined to the side in a sort of beak, or even hook. It is the classical figure, which has interest, not only because it is thus the spermatozoon is seen in life, but especially because those of animals do not have the pyriform aspect in this position. They are seen as a small rod, lightly attenuated at the two ends. This is an excellent distinctive characteristic to which I will return.

Insertion of the flagellum is not in the axis of the head, but encroaches on one face, like the handle of a spoon; I have said a word about the brilliant point seen there. The insertion seems *almost articulated*; in reality, there is no cavity, as in the dog, but most often (I am discussing sperm from stains) a slight protuberance ending in a facet, which is not always as pronounced, however, as appears on one of the isolated heads.

The tail or flagellum of the human spermatozoon has been described for a long time as a simple thread. This is effectively how it presents itself in ejaculated semen, if examined with the strongest magnification, and even after the action of coloring agents. But the fact that this flagellum is capable of movement already demonstrates that it cannot be homogeneous, and that its structure is complicated. It is to the works of Dujardin, Henle, Grohe, Koelliker, Ankermann and Schweiger-Seidel for an initial period going up to 1865 that is owed the first notions of the histology of the tail of the spermatozoon. The last of these authors had already at this time distinguished a *head* (and in certain cases a *hat* (Kopfkaffe), a *middle segment* (Mittelstück) and a *tail* (Schwanz). The middle segment had as its essential characteristic that it was cylindrical, i.e., the same thickness in its whole length, to have a particular luster and a constant length in each animal species. Schweiger-Seidel gives the following table which reports the respective lengths of various animals.

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Average length in millimeters

	head	middle segment	tail
mouse	0.008	0.023	0.085
hedgehog	0.006	0.009	0.065
"maulwuey"†	0.008	0.020	0.055
guinea pig	0.012	0.010	0.080
rabbit	0.007	0.008	0.041
ram	0.008	0.015	0.055
pig	0.009	0.011	0.040
"inuus"†	0.006	0.011	0.043
man	0.005	0.006	0.040

† [meanings could not be decided, nor could the original articles from which the table is said to have been taken, be located].

Dujardin (*On the zoosperms of mammals. Ann. nat. vol. VIII, 1837*) was the first, I believe, to have studied the accessories of this middle segment; a little later, this study was resumed in Germany, where *nodules* (*Knotchen* of

Schweiger-Seidel) and strips (*laeppchen*) were described. Grohe at first, then Schweiger-Seidel, thought they saw two small cavities (Lucken, Vacuolen, Hohlungen of varied authors) serving as a sort of hyphen between the middle segment, the head and the tail. The last author gave a design which clearly represents these two cavities, and on the same plate are spermatozoa whose middle segment presents curious transverse striations, which the author considered as phenomena of alteration. He remarked, in addition, that during the movements of progression of spermatozoa, movements which from that time were the object of much research, the middle segment, as well as the head itself, remained absolutely passive.

The second period was marked by Eisner in 1874 (*Untersuch. über den Bau der Samenfaeden. Verhandl. der phys. med. geo. Zu Wurzburg (vol. 6. 1874)*). This author discovered what he called the *Centralfaeden*, a name which Brunn replaced with *Axenfaeden*, axial filament. This filament traverses the flagellum of the spermatozoon in its entire length, in a state of extreme tenuousness, Eisner remarked, without being too certain of it. He presumed that the end of the tail (or tail proper) must be formed exclusively by this filament, whereas the middle segment was made from small cubes strung on the filament like little pearls. In 1879, Henle and Gribbes (*Quart. journal of micro. science, vol. XIX, 1879 and XX, 1880*) discovered a spiral thread which made six or seven turns around the middle segment in mammals, as had been seen long before in the salamander. Jensen confirmed this discovery (*Die Structure der Samenfaeden, Bergen, 1879*) and asserted that this spiral thread is specific not only to the middle segment, but is found also on the third segment, the tail, and pointed out that the filament has a different chemical constitution from that of the spiral thread in the middle segment, but not in the tail, where the two filaments behave the same in the presence of reagents.

Retzius (*Zur Kenntniss der Sperm. Biol. Untersuch. 1881*) finally discovered that the third segment, the tail proper, is itself formed by two distinct parts, one he calls *Hauptstück, the principal piece*, the other, *Endstück, terminal piece or terminal filament*. He called the middle segment of Schweiger-Seidel, *Verbindungstück, joining piece*, and believed that what was described as a spiral thread was only a sort of spiral hem around the flagellum. Since then, this question has impassioned anatomists. Brunn (*Archiv. f. mikr. Anat. B. XXIII, 1884*) carefully described the fine, regular transverse striations of the middle segment in the mouse that Leydig (*Unters. Zur Anat. und Hist. der Tiere, 1883*) had already acknowledged as a spiral thread, but Brunn could not determine if he was dealing with a spiral thread or simply transverse striations. Kraun (*Der Spiraalsaum der Samenfaeden. Internat. Monatsch. f. Anat. Hist. Vol. II, 1885*) was more assertive, and Plattner (*Über d. Spermatoz. bei den Putmonaten. Archiv f. Mikr. Anat. Vol. XXV, 1885*) observed, in his turn, that the middle segment in bulls is spiraled, thus verifying the views of Jensen and Brown (*On spermat. in the rat. Quarterly Journal of*

*Microsc. Science*, Vol. XXV, 1885). In treating spermatozoa with gold chloride, he observed a dark, spiral thread which surrounds a little-colored internal substance. Furthermore, Plattner seems to have seen two threads; in any case, one of his designs (Pl. XXIII, Fig. 18) indicates two.

Up to then, no one had seen the unrolled spiral thread. Jensen, at first, Ballovitz, Prenant of Nancy<sup>5</sup> and others after them, proved its existence without doubt. According to Jensen, it suffices to collect spermatozoa from rat testicle, and simply add to them 0.6% saline solution—and even without this addition—to clearly see a regular striation, where each stripe is separated from its neighbors. Toward the end of the median segment, these stripes separate more and more such that they become more and more oblique in relation to the axis of a segment. If the tube of the microscope is lowered, to focus the deeper parts, it is seen that below, the stripes have an inverse obliquity, which proves that it is a spiral thread, and not rings, forming these striations. Putting the spermatozoa in aqueous glycerine (at  $\frac{1}{6}$ ) the spiral thread separates. Acetic acid at 1% also isolates it, but this reagent has a destructive action and soon fragments the thread; in certain animals the destruction is slower, and the curious action of this reagent can easily be observed.

Whereas the spiral filament is homogeneous, of fine, clear contour, very slightly refringent, the axial thread (or “axe file”) is thicker, very refringent, terminating toward the head in a small button even more refringent again, the spiral filament beginning just after the button. Ballowitz, who has performed lengthy studies on this question, established that the axial filament is formed by two fibrils, each ending in a small button, and he could sometimes observe three or even four, on sperm taken from the epididymis. In the rat, this axial filament shows itself as a packet of twisted wicker, and he could count up to seven well-isolated fibrils in the middle segment. He concludes from his research that this axial filament is formed by two bundles of fibrils joined by a cement, traversing the whole spermatozoon and appearing in the free state only at the neck and tail.

In summary of all these works, the spermatozoon is formed by a head and a flagellum; its complete length is traversed by a complex axial filament, which is uncovered at its union with the head where it forms the *neck*, and at the extremity of the tail (*Endstuck*). This total length is divided first into a *middle segment* (to which is suited the name “body”, it seems), cylindrical, formed by a spiral thread rolled in a certain number of turns; then into a *tail* proper, divided into two pieces, the first, principal piece (*Hauptstuck*) which tapers, is also constituted by the rolling of a spiral thread around the axial filament, then this, existing uncovered from the principal piece, forms the *terminal filament* (*Endstuck*). A curious appendix is inserted in the middle segment; it is the coat, the protoplasmic coat, in the form of a thin, granular, transparent membrane, inconstant in the mature spermatozoa. This membrane is sometimes in

an irregular strip, sometimes in the form of a trumpet directed toward the head and encroaching on it.

Up to now, alas, all these interesting notions have not been able to be used in legal medicine, because these delicate structures are only visible in sperm from testicle, the epididymis, or *vas deferens*. As soon as the sperm is ejaculated, the spiral filament is welded so strongly to the axis that all is perfectly homogeneous; this is the opinion of Brunn, and Jensen is not far from agreeing. However this point of view cannot be accepted, for the spermatozoon, having reached maturity, is endowed in its flagellum with rapid movements, which necessarily implies a persistent fibrillary state; a perfectly homogeneous mass could not be endowed with movements in its parts. I do not despair in finding a reagent, not to see all the details of structure which I have just presented, but strong enough to permit at least recognition of segments, for these are not of the same chemical nature. In heating dry semen, kept on a glass slide, to 40° for a few hours in a humid room, and then in treating it with saturated solution of crocein, I have seen the middle segment of separated tails enlarge considerably, curve itself and clearly present the axial filament, having the appearance more of a fine cavity than of a thread. Then the incurvation gradually continues, in proportion to the thickening of the segment, and its two ends finally join and weld together. This strange phenomenon takes more than an hour to finish; the segment then presents the appearance of a disc with a central depression and a very fine circular streak, representing the axial filament, in the middle of the radius.

Moreover, the protoplasmic coat is often found, whose resistance to chemical agents is considerable; next, what Schweiger-Seidel described under the name of *nodules*, and which are only forms of alteration of the middle segment, or all the elements of the spiral filament which are visible. Frequently in sperm extracted from stains and treated by crocein, these small swellings of vague contour, four, five and more in number, generally of unequal size, are found on the segment. These small successive swellings, which give a winding appearance to the spermatozoon, have been described by Robin.

*to be continued*

#### References and Notes

1. See the general bibliography at the end.
2. I find this name written in various ways.
3. This hypothesis is not absolutely implausible. It was upheld in 1836 with brilliance by Sielold, then by Fraisse, and recently in a masterly article, Brunn demonstrated that *Paludina vivipara* has two sorts of spermatozoa. (*Archiv. f. microsc. Anatomie*, 223, p. 413)—My friend R. Koehler also described the two forms of spermatozoa of *Murex brandicus* and *trunculus* (*Comptes rendu*, 1888).
4. There exist many different agents which, in the trade of stains called “aniline,” bear this name; the crocein I use is furnished to me by the maison Stéphan Girard, of Fontaine-sur-Saone.
5. Note on the *Structure des spermatozoïdes chez l'homme*, 1888, and Rem. on the *Structure des sperm.* (*Revue du Nord de la France*), Lille, 1888-89.

## Semen and Seminal Stains in Legal Medicine \*

(Sequel)

Dr. A. Florence

Second Part

Technique of examination of stains

/37 **History.** It seems to me quite difficult to admit that past experts didn't make any use of confirmation of sperm in cases of rape or of impotence, yet I found practically no proof of it, for various reasons: the library at the law faculty in Lyons, recently founded, is relatively poor in old documents of this type; that of the medical faculty, on the contrary, has quite a large number of old treatises on legal medicine, all of which I perused with great care, but without finding one single fact where a seminal stain played any role; most often, the word was not even pronounced. The reason is that up until recent times intervention by respectable matrons was considered much more appropriate in affairs of this type than by physicians, and, besides, much experience was readily attributed to them in this kind of thing. A decree of Innocent III<sup>1</sup>—this same pope who was the first to recommend the examination of the corpse by a physician in murder cases, and introduced this custom into canon law—confided to these matrons the examinations concerning impotence. It was they who sovereignly judged in the proof of sexual union, and it is known that they had to determine if there had been intromission, *et an fuisset emissio, ubi, quid et quale emissum.*<sup>2</sup>

/38 Unfortunately they have left us no report on the procedures they followed. It's a shame, for it must certainly have been quite interesting.

During the first period of the Middle Ages, the violated woman presented herself at the court with her torn clothing, and showed the very traces of the awful treatment to which she had been submitted (Pardessus, *Commentaires de la loi salique*, p. 567).

The ancient Fueros of Spain admitted the intervention of matrons: "In regard to this woman who complained of having been forced, if the act took place in the fields, she had to throw down her cloak in the first city encountered, and lie on the ground saying: Such and such a man forced me, if she knew him; if she did not, she gave some information about him. If she was a virgin, she must show proof of rape to the most reliable woman she could find . . . if she has not thusly

acted, her complaint is not whole, and the accused can defend himself." She could also prove the crime by intervention of two men, or by one man and two women of honor. (A. du Boys, *Histoire du droit criminel en Espagne*, p. 136). This dramatic procedure, where the violated woman presented herself with her tears, her torn and bloodied clothing, endured for a long time, and it was not until Alphonse X, that she could bring her complaints to the steward of the king (*Idem*, p. 399).

In France, in response to the ridicule thrown at the congress in the famous suit by the marquis of Langeais (in 1659), who, even though declared impotent, had seven children by Diane of Navailles, the Grand Chambre definitively abolished this singular procedure. But the role of the matrons was not stopped by that: on the 25th of June, 1707, a decision of the sovereign council of Alsace enjoined persons declaring themselves to have been *forced or raped* to appear before the matrons.

Much closer to us, Jousse, an authority on these matters for a long time, indicated, in 1771, admissible proof of violence done to a woman or girl.

These proofs, he said, derive from:

1) The testimony of witnesses who saw the violence.  
2) Circumstances of the fact, e.g., if the woman's cries were heard, in relation to the violence being done her, or if she was heard to cry for help in a remote place, where the voice made itself heard with difficulty, especially if the person claiming to have been violated is of good reputation (Julius Clarus).

3) But the sole declaration of the girl who asserts that one has done violence to her is not sufficient proof (Julius Clarus). All the more reason is she not credible if it is proven that, since the rape, she voluntarily abandoned herself to him or that she leads an ill life.

4) Boérius holds that a young girl is not presumed violated if pregnant(!)<sup>3</sup> (Jousse, vol. III, p. 751; 1771). It is quite necessary to admit that the jurisconsults of that time had profited very little from the words of Voltaire.<sup>4</sup>

It is evident that no one imagined making use of the confirmation of sperm, for even the signs of deflowering did not intervene, even though perfectly known; but it was just as well known that a woman could have been deflowered without appearance of these signs, and also "that she could have her virginity with the supposed marks of deflowering".

\* Translation of: "Du Sperme et des Taches de Sperme in Médecine Légale". In *Archives d'Anthropologie Criminelle de Criminologie et de Psychologie Normale et Pathologique* 11: 37-46 and 146-165 and 249-265 (1896). (Contains the second, third and fourth parts of the article, the first part of which appeared in the same journal, vol. 10, pp. 417-434).

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medicine had drawn so much mistrust in assessments that it was preferred to forego it. Stains were, likewise, totally neglected for many years, and even though Sue, professor of legal medicine at the Faculty of Paris since the year VII had done an ingenious investigation on blood stains, Orfila did not say a word about it in his celebrated *Traité des poisons*, 2nd edition in 1818.

In his *Leçons de médecine légale*, published in 1823, he treats procedures which must be used to determine if the confirmed deflowering is the result of introduction of the male member or another body; he does not indicate the confirmation of semen in the vagina of the victim; nor does he speak of it when he considers death attributed to rape. He is forced most often to leave the question unresolved as he does in assessments 1, 2, 3 and 4 which he reports, assessments  
/40 where the most elementary examination by microscope would have peremptorily resolved the question. Finally, in the case of impotence in a man, he did not have recourse to the microscope. Of the rest, Poilroux, in his *Médecine légale* published in 1837, does not behave otherwise, and it is only in his 1848 edition of his *Médecine légale* that Orfila finally indicates the confirmation of spermatozoa in cases of this type.

In 1826, Ollivier d'Angers and Barruel had to examine stains in an affair of rape (*Journal de Chimie Médicale*, 1826, p. 565). These experts heated the stains, which were rose, and determined that they emitted no odor of fat. They moistened it with water, and rendered it milky by shaking; alcohol, added to the maceratum, clouded it even more. The experts then concentrated the liquid, and noted that it turned litmus paper blue; after total evaporation, the liquid left a yellowish residue, and gave a strong animal odor on calcination. The authors concluded that the stains *could have been* the result of the applicaiton of semen to the surface of material due to three characteristics of the spermatic liquid:

- 1) being partially soluble;
- 2) leaving a residue which made the material sticky;
- 3) being alkaline.

At this time, numerous experiments were attempted to try to find spermatozoa in the stains, notably by Orfila, but without success; this was undertaken without much hope, for the spermatozoon was generally considered as ubiquitous, as some type of infusoria. The main experiment of Spallanzani who had demonstrated that filtered frog sperm (and consequently supposed free of spermatozoa), and much older of Hartsoecker, who, a little while after the discovery of the animalculi, found that semen obtained after numerous ejaculations, in which he had not observed spermatozoa, would produce fecundation, were still the authorities in the science, despite the beautiful work of Dumas and Prévost. (1824).

Animalcules whose imperfection hardly permitted them to be distinguished from spermatozoa, were also observed; their origin, however, had nothing in common with sperm.  
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Thus, in the dictionary of Medical Sciences of Panckouke, in the article *Sperme*, appearing in 1821, the animalcules are claimed to have been found in liquids other than human

semen, and it is claimed that Spallanzani fecundated frogs with semen without spermatozoa, and this sufficed to destroy the system of Leeuwenhoeck. Likewise, the article *Génération*, appearing in 1817, points out that cercaria found in semen appear extraneous to fecundation, contrary to the opinion of Leeuwenhoeck, of Hartsoecker, of Valesnieri.

The belief in *aura seminalis* endured for a long time, even with forensic physicians. And it is not astonishing that Dervergie in 1839 wrote the following observation concerning the examination of the semen of two brothers, both without children, and of which the spermatozoa (?) were of a peculiar form, ovoid corpuscles presenting movement:<sup>5</sup> "If analogous facts in sufficient number were observed, one would perhaps be able to enlighten the question of the cause of fecundation: to determine whether it is accomplished by means of spermatozoa or if, on the contrary, the hypothesis of an *aura seminalis* has some basis."

Orfila presented his research on seminal stains to the Royal Academy of Medicine, at its meeting of Aug. 25, 1827, and concluded it is not possible to find cercaria or animalcules in these stains by microscope. The same year (*Journal de Chimie Médicale*, vol. III, 1827) his important article appeared: *Semen considered from the medico-legal viewpoint*. The illustrious scientist was called to give his opinion on the report of a physician who, on examination of the sexual organs of a young girl of 13 years, 9 months, who had allegedly been raped nine days beforehand, concluded that the rape had been consummated and claimed to have retrieved a certain quantity of semen from the vagina. Orfila, observing that science possessed no adequate means for facilitating the solution to the problem, affirmed that it was unlikely that semen had remained nine days in the vagina of this girl who was afflicted with a mucus discharge, and the accused was acquitted. This assessment convinced Orfila that he should concern himself with the methodical investigation of the diagnosis of seminal stains. Being aware of the physical characteristics, he indicated the following procedures as a technique of examination:  
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1) Sperm stains brought close to a flame become a tawny yellow and this tint disappears if the stain is immersed in distilled water for several hours. This was, for Orfila, the most important sign.

2) The stains moisten fully, which does not happen to fat stains;

3) The stains macerated and pressed by a stirring-rod become viscous and *emit a spermatic odor* when compressed between one's fingers;

4) The liquid, filtered and evaporated at a very low heat, becomes alkaline; it shows during evaporation the viscous appearance of a solution of rubber, does not coagulate, but deposits a few glutinous flakes, and its consistency becomes so particular that it is difficult not to accord an importance to this characteristic;

When dried, it leaves a semi-transparent residue, shiny, of a tawny color. Put in water again, this residue separates into



two parts; one glutinous, yellowish grey, adhering to fingers like glue, insoluble in water, soluble in potassium; the other, soluble in water.

5) The solution gives a white, flaky precipitate with chlorine, alcohol, lead acetate, lead subacetate, and mercurous chloride; *pure, concentrated nitric acid* gives it a light yellowish tint, if it is uncolored, but does not cloud it, whereas all the other morbid vaginal discharges become cloudy. Alcoholic tincture of gall-nut gives an abundant greyish deposit.

Orfila, speaking of microscopical examination, adds: "It is easily understood that no benefit can be derived from microscopical observations for the recognition of stains. The animalculi are not more appreciable if after drying the semen on material, it is diluted in water for examination by microscope." But he remarks that spermatozoa coming from stains on glass are more visible and he claims to have "*recognized them perfectly in semen dried for eighteen years*". Orfila does not seem to accord a great confidence to the verification of spermatozoa, for he says that "the existence by itself of animalcules of this form (and executing very marked movements) in the extreme case permits the attestation that the *solution* submitted to examination is semen, since no other liquid is observed *with these characteristics*. However the physical and chemical properties which I have already mentioned *must be looked for in this solution*."

This page, in which Orfila showed justified prudence, was strongly reproached in 1839 by Devergie in the violent discussions they had on the subject of priority of the use of a microscope in examination of these stains.

In 1834, Chevallier had to examine suspected stains. He did not use the microscope, but operated, in following Orfila's procedures a little, by comparison with seminal stains; he was not pleased with these procedures, especially the action of heat, of which was made great account, having found nothing clear and he was prudently forced not to reach any conclusion.

Devergie did an assessment in 1834 (*Médecine Légale*, 2nd ed., 1840, p. 387) and relied on the yellow color and absence of the smell of burning which the heated stain presented; on the spermatic odor which developed only the following day; on the starched state of the stains after washing with water, dessication, and finally nitric acid. These are the only procedures indicated in the 1837 edition, vol. II, p. 181, of his *Médecine légale*, and they scarcely differ from those of Orfila.

These investigative procedures were a great success, and were practiced for a long time, especially in foreign countries; thus, in the *Praxis* of Fredreich (1855), the author at first presents the procedures of Orfila, then for just as long, those of Devergie, but, something curious, in this very important book, which had several editions, he did not say a word about microscopical research of spermatozoa in the edition (2nd) of 1855, where Friedreich devoted numerous pages to the study of stains.

In 1838, Devergie read a very important memoir entitled: *New signs of death by hanging*, at the Academy of Medi-

cine, in which he used a microscope to confirm the existence of spermatozoa in the urethral canal of those hanged. Before this, he had established, in the affair of the murder of Tessier, in collaboration with Turpin, that no act of pederasty had taken place, for there were no spermatozoa in the urine emitted by the victim before death.<sup>†</sup> Finally, Devergie announced in this memoir that he had *been able to confirm spermatozoa in semen stains existing for ten months on cloth, a fact that much more important, since the means provided by chemistry for recognizing stains don't have all the certainty one has the right to expect in a medico-legal analysis*. It is, then, Devergie, and not Bayard, as one often writes, who deserves the merit for this discovery. But Devergie had been preceded by almost twelve years: in 1827, Lassaigne, having recounted to Chevallier that he had been the first to retrieve spermatozoa from a stain, Chevallier informed him that Lebaillif, in the affair of the rape of Contrafatto, had determined the nature of seminal stains in this way. Lebaillif did not publish his report, but there was no doubt as to the priority of his discovery: he had a great reputation as a micrographer and all studies of this type were addressed to him. It was thus that Orfila committed himself to find a method for determination of blood stains by microscope (*Journal de chimie médicale*, 1827).

In March, 1837 (*Journal de chimie médicale*), Ratier, in macerating materials stained with semen in watch glasses, and in submitting the liquid to microscopical examination, succeeded in retrieving the spermatozoa and pointed out on this subject the advantages which legal medicine might derive from this mode of investigation.

In 1838, without acquaintance of Ratier's note and even less of the appraisal by Lebaillif, which, not having been published, remains a dead issue, and before the memoir of Devergie had appeared, Bayard deposited at the meeting of the Society of Hygiene and Legal Medicine his beautiful work entitled: *On the Use of the Microscope in Legal Medicine*. (*Annales d'Hygiene*, p. 134, 1839). It is not an attempt, a simple affirmation, like that of Ratier, who had never made an appraisal of stains, who undoubtedly succeeded one time in isolating spermatozoa, but who would have perhaps not succeeded a second time, and who certainly did not isolate blood cells from a stain with the impossible procedure which he indicates in the same note. It is a conscientious methodical study, devoted as much to demonstrations done in the presence of members of the Society of Legal Medicine, as to *eleven assessments* done with brilliance. And, there is some merit, if not courage, in disputing the validity of the categorical assertions of Orfila. And the fact that successful attempts were done before his, undoubtedly unknown to him, is no reason to refuse to Bayard the merit of his important discovery. If there were workers of the first hour, it is he, and only he, who was architect of the edifice. It would be, however, an injustice to forget a few scientists

<sup>†</sup> [victim was a woman].

who, on the whole, inspired all this research and brought it into focus by their works: I speak of Dumas and Prévost, and especially of Donné, whose names must be indissolubly tied to the history of determination of stains.

Following the work of Bayard, and due to the polemic waged between Orfila and Devergie, there appear in Paris, from 1837 to 1839, ten theses on the determination of stains: unfortunately I could not procure them in order to complete this story.

**The Bayard procedure.** In the beginning, Bayard simply macerated in distilled water the strips of stains, which he took great pains not to rumple, and whose fibers he did not dissociate. After several hours of immersion he lightly heated without boiling them, then examined the liquid. Not very satisfied with the results, he indicated his *second procedure*:

1) Cut out with scissors and remove with care a portion of the stains presumed seminal; do not rumple the material, and place in a test tube;

2) Bathe the stained material in distilled water, and macerate for twenty four hours;

3) At the end of this time, filter the first liquid. Place the stained material, already macerated, in a porcelain capsule, moisten it with distilled water and heat by the flame of an alcohol lamp until the liquid attains a temperature over 60° to 70°. Filter this liquid. Finally treat the stained material with alcohol or by ammonia solutions and filter the diluted solution;

4) When the filtration is finished, cut the filter paper at a distance of one thumb from the edge and turn it over on a watch glass, or, preferably, on a dish of flat glass; moisten the inverted filter with alcohol or ammonia solution, which dissolves mucus and entirely detaches the deposit. If fatty matter is found mixed in, a few drops of ether water is employed. Microscopical examination of the capsule or the dish of flat glass permits recognition of *entire spermatozoa, without breakage* of the tail, isolated from mucus.

Alcohol in water was 1:10, sodium and potassium 1:20, ammonia 1:16, and Bayard made observations at a magnification of 350 to 600 diameters.

What comes out of the beautiful work of Bayard, is that these are things *seen* which he describes, and he has performed a great many times his conscientiously presented experiments. I will point out as proof of the sincerity of his observations only two facts: p. 156, he remarks that if the preparations are dried, the spermatozoa are more visible, an observation later made by Schweiger-Seidel, and, more recently, by Pincus, who made of it a procedure he believed new; then, p. 161, he described the crystals of semen, which he takes to be sodium and ammonium phosphate, crystals known today under the name of *Charcot crystals*. It is then, he who deserves the merit of the discovery of these crystals and not Robin, nor Charcot.

146 The Bayard procedure, that I will call the filtration procedure, seems to have been followed for a long time despite its difficulties; the author reproduced it without changing

anything in his *Manuel de medecine legale* of 1844. Yet in the six-year interval numerous works had appeared on this question, particularly in the form of theses at the Faculty of Medicine in Paris, as I have already noted.

In 1848, C. Schmidt indicated his squeezing out procedure, which has as its principal advantage to leave the clothes intact, which can be of importance in discreet examinations. The material is examined with care to assure which side holds the stain. In holding it obliquely, it is easy to see one of the faces more marked than the other and it might even show a projection; then, taking the stain as center, the material is folded in a point or cone, with the stain forming the *outside* peak. This point is then suspended vertically over a watch glass half-filled with water, into which it scarcely dips. It is left in this position for three or four hours, replacing the water as needed; then this is slowly heated by means of a small spirit lamp. A few drops of ammonia solution are added, and the material is picked up, pressed from the top toward the bottom *between the thumb and index* finger, the stain is thus expressed, it disappears, and the water flowing out is cloudy and a bit mucous. It is examined by microscope. This procedure, on the whole, quite simple, perfectly succeeds when the stain is rich, but in difficult cases, in which it is hardly visible, nothing is obtained, or at best, a few fragments. (C. Schmidt, *Die Diagnostische verdaechliger Flecke in criminalfoellen*, Leipzig, 1848, page 42). This procedure is most often followed by experts of the Russian Ministry (*Anleitung zur Untersuchung verdaechliger Flecke*, etc. Saint Petersburg, 1871).

Koblank<sup>6</sup> in 1853 (Casper's *Vierteljahrschrift*, III, p. 140) gives another squeezing out procedure which enjoys a certain favor undoubtedly due to the support of Casper. The stain, cut out, is put in a saucer containing a little cold water; the material is dipped in the liquid with a stirring rod until it is completely saturated. After a quarter hour, a drop of this water is observed under the microscope; the presence of spermatozoa is easily determined. The material must be pressed with the stirring rod.

If the stain is complex (blood, fecal matter, etc.) and the liquid too cloudy, Koblank recommends adding a few drops of acetic acid which clears the preparations, without affecting the spermatozoa. This procedure, less brutal than the preceding, perhaps better safeguards the integrity of spermatozoa, but it gives absolutely no *result with very meager stains*.

**Scraping procedure** of Ch. Robin and A. Tardieu (Memoir on a few new applications of microscopic examination to the study of various types of stains. *Annales d'hygiene et de medecine legale*, 1860, p. 434).

The authors cut out the stains, macerated them to saturation in a watch glass, then scrape them with a bistoury. They find spermatozoa "as numerous and concentrated as in spermatic liquid, as it is found when it has just been ejaculated: they are whole and flexible, and there was a very small number which were broken." They add a little acetic acid to dissolve mucus substance. The two experts, though having a

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peremptory proof of the seminal nature of these stains, did not think themselves dispensed from submitting them to chemical reactions, such as Lassègne had indicated, i.e., the action of heat, of cupro-potassium subtartrate, and nitric acid. It is true that, little satisfied with the results obtained, they add: "These characteristics, to which a few authors still attach a certain importance, have none, however, beside those derived from the presence of spermatozoa". This scraping procedure is assuredly the simplest, the most rapid, and the easiest of all; we do not hesitate to employ it when the stains are thick. Professor Renaut had formerly supported it with his authority.

But if the stains are meager, it is an absolute disaster, with a high risk of losing it all, while with a less brutal procedure, one would have succeeded perfectly.

Up to this time, the exclusive preoccupation was the *extraction* of spermatozoa, without indicating a procedure to see them better, either by staining, or otherwise. Pincus, having had to do an expert investigation, found nothing in his preparations, but, in looking at them the following day, when they had dried, he saw a great number of them in a perfect state (*Vierteljahrschrift für Gerichtliche Medic.*, 1866, N.F., Vol. V, p. 347). This caused quite a commotion, even though it was nothing new, for Bayard had indicated it in France in 1839, as I have already noted, and besides, Schweiger-Seidel in Germany had also brought attention to the advantages of letting spermatozoa preparations dry before observing them.

This skill was greatly reproached, for it is only a skill and particularly by Ungar. It is, however, certain that if a spermatozoan is not dried out, but if the liquid surrounding the preparation has disappeared, it is in the best possible conditions for being observed, whether stained or not; it is bordered by a little meniscus due to capillarity, a strongly refringent meniscus which gives an extraordinary relief.

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Thus when a spermatozoon accidentally finds itself lodged in an air bubble, or if the liquid is aspirated with precaution by blotting paper, the end of the tail is perceived with perfect clarity, something very difficult to delineate exactly without this method, at least without a special utensil. Unfortunately, it is of little convenience to wait until the following day to observe the preparations, since all the particles floating in the liquid, spermatozoa as well as the rest, are dragged in a heap during dessication by the meniscus which is pulling back, and everything reaches the border of the thin slide. Here is the danger, for in the accumulation of the amassed material in one place, it is very difficult, if not impossible, to find the rare spermatozoa.

On aspiration with blotting paper, one runs the same risks which can be partially avoided, in strongly compressing with the paper *only the edges* of the upper with the lower slide in such a way that water is always able to pass, and the solid particles disperse according to size in the sinus formed between the two slides. But all this becomes obsolete due to coloring.

Roussin in 1867 was, I believe, the first to have the idea of

staining spermatozoa to facilitate their visualization; he was also the first to substitute methods employed up to the present with his *unraveling procedure*, the only one permitting success in every case. I cannot praise too much the remarkable memoir of this able and conscientious expert, who made an immense step forward in this delicate research. We quite often follow his procedure at the laboratory of legal medicine at the faculty of Lyon in the way he described it. I will present it further on. As for his staining solution, it has stood the test of time and has emerged victorious from all attacks. The solution contains:

Iodine .....1  
Potassium Iodide .....4  
distilled water.....100

"This reactant", said Roussin, "alters neither the volume, nor the form, nor the external texture of the spermatozoa, which suddenly take on a remarkable relief on contact, and are separated in the field of the microscope with the greatest clarity. The clearly visible portion of the tail increases considerably<sup>7</sup> and the whole preparation takes on a precision difficult to define<sup>8</sup>". All of which is quite exact. It is only with difficulty that I can account for Hoffman<sup>9</sup> finding no advantage to this coloring, under the pretext that the whole preparation was uniformly colored; Ungar<sup>10</sup> contends that phenomena of coagulation produced by the liquid, in engulfing all the mass, most often hinder finding spermatozoa, and consequently go precisely against the end to be attained.

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We often have recourse (M. Lacassagne, M. Contagne, and myself) to the liquid of Roussin, and we have had to make a similar reproach; it is true that we always employ it at the dilution indicated by Roussin, and as we put only a small drop on the preparation, the dilution is thereby reduced to 1/200, while in Germany it is employed, due to misprint no doubt, sometimes one tenth (Maschka, vol. III, p. 126). As to the rest, when the spermatozoon is isolated and perfectly colored by the reagent of Roussin, it is less of a hindrance than one would think that other foreign bodies of the preparation might be equally colored; the eye knows it, is attracted by it, if I might thus express myself, and seizes it in passing when the preparation slides before it.

This reagent admirably fulfills the principal goal, to leave absolutely no doubt in the mind of the expert once the spermatozoon is found, which always comes with patience; it is of little bearing that one looked a bit more or a bit less, the essential thing is that no error can be committed with regard to the nature of the object found and I strongly affirm that in this context, the liquid of Roussin is perfect. It stains the external envelop of the head of the spermatozoon, which is admirably distinct from the background, with vivid and clear-cut contours, and if an objective of higher power is then substituted, there is no possible doubt.

Longuet proposed in 1876 to substitute ammoniacal carmine for the liquid of Roussin which he also reproached for staining everything.

He absolutely rejects the unraveling method used by

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C. Robin and Tardieu which he accuses, among other misdeeds, of *artificially creating the spermatozoa*.

As strange as it appears, this accusation certainly has some basis; by the brutal unraveling are freed fibrils of material and also granulations which Longuet claims peculiar to hemp<sup>11</sup> which can be perfectly confused with the heads of spermatozoa.

It is common that these so numerous fibrils juxtapose themselves on a granulation to closely simulate a spermatozoon, especially if observations are done with the weak magnifications generally used. All those who have had to examine stains are able to make this observation.

Longuet indicates that this error can be avoided in noting that in false spermatozoa, the tail has a diameter equal everywhere whereas the real tapers toward the extremity. One will see further on other procedures which leave less room for error.

Longuet estimates that maceration must be prolonged forty-eight hours, which, he said, is without inconvenience, due to the extraordinary resistance of spermatozoa, which even ammonia steeping does not destroy. It is only after this time that the spermatozoa have regained all their suppleness and that the unraveling of fibers does not break them. As to the rest, a long maceration is certainly necessary, if one wishes to follow the indications of Longuet, because ammoniated carmine tints spermatozoa rather poorly and slowly, even after forty eight hours; in contrast, says the author, it leaves intact all vegetable elements, fibrils or granulations, "one sees so well at first glance that all which is white is vegetable and all which is become red is of animal nature". The reagent of Longuet is formed from five to six drops of ammoniacal carmine solution, such as is used in laboratories, in five grams of distilled water.

In the following year Petel and Labiche gave a procedure also based on the use of ammoniated carmine, but with the considerable difference that the determination of the nature of the seminal stains passes the microscope by completely, and can then be applied to cases where spermatozoa have completely disappeared as a result of breakage. Suspected stains treated with ammoniacal carmine strongly color in red, and afterwards energetically resist washing with pure water or even more than twelve hours in a solution of sodium carbonate. All the other stains by contrast were discolored in less than six hours according to the authors.

I did not find the formula for the solution of carmine employed by Petel and Labiche anywhere, which is, moreover, immaterial, but what is not at all immaterial is the concentration of sodium solution, which plays here a major role. The Society of Legal Medicine charged two of its most distinguished members, M.M. Brouardel and Boutmy, to report on this procedure (Meeting of May 12, 1879). One reads with great interest in the Annals (1880 p. 225) the remarkable account rendered by these two able practitioners who, though acknowledging the benefits able to be drawn in certain cases by this coloring of stains, quite wisely pointed out how imprudent it would be to base the condemnation of

a man solely on such a frivolous sign, a sign still uncertain; where, in short, the basis is but a matter of more or less about six hours to twelve hours.

It is then absolutely wrong when Vogel says that M.M. Brouardel et Boutmy confirmed the assertions of Petel and Labiche (*Viertel. f. ger. Med.* 1882, p. 160, Boutmy and Brouardel, *bestaetigen dieses Verhalten*). The contrary would be more exact, it seems to me. Vogel criticized the procedure, and said he could establish that varied stains, of vaginal mucus, or white flowers, discolored like those of semen, as much in a concentrated solution of sodium carbonate as in a dilute solution. He ends by saying that the procedure can at best serve as negative proof and demonstrate that a stain which refuses to color itself with carmine is not formed by semen. But, he adds, a long time ago Hager (*Untersuchungen*, 1871, p. 461) indicated picric acid for this same end; on the other hand Hager very prudently remarked that many other stains behave like semen, for example those of vaginal mucus, eggs, flour, nasal mucus, an important fact, he said "because it is a very common habit of the women of the population to blow their noses in their shirts(!)"

**Destruction Procedure.** Vogel (*loco citato*) found fault with the procedure of Petel and Labiche and also that of Longuet, which he reproaches for staining especially the other elements of semen, precisely for leaving intact and uncolored, even after fifty-four hours of maceration, the spermatozoa themselves. These scarcely have a little bluish tint due to a phenomenon of refraction. In the face of the insufficiency of these procedures, Vogel indicates one which is certainly unexpected. Discontent with all stains: picric acid, aniline blue, Methyl violet, picroaniline, fuchsin, eosin, Bismarck brown, etc., he simply destroyed the support of the stain, or its debris, in respect to the spermatozoa. The stains, he said, are moistened with water, then scraped by knife, taking care to remove only the least possible material, but a few threads are no problem, for they will be destroyed. On the bottom slide, he adds concentrated sulfuric acid to the product of the scraping, then after two minutes, one or two drops of tincture of official iodine. He stirs softly with a stirring rod and puts on the top slide. All is destroyed except the spermatozoa, which are vividly colored in brown by the tincture of iodine. Unfortunately, the preparations, as one might suppose, keeps hardly two days at best, even after washing. It is assuredly abusing the resistance of spermatozoa to attack them with the most violent of our corrosives, uniquely to destroy a few fibrils which can hinder the research, but to which with a little practice, one pays almost no attention. Moreover, the considerable heating produced by the mixture of the water of the preparation with concentrated acid can compromise everything in certain cases.

**Staining with Eosin.** Ammoniacal carmine was generally unsatisfactory, staining inconsistently, sometimes rather well, other times very badly, and eosin was accepted with great favor in all the laboratories, as its manipulation is particularly convenient. It always instantaneously stains

spermatozoa in a splendid, vivid rose; the head especially is remarkably discernible, and takes on a lively refringence under the influence of the reagent. It is generally accepted that Schnitter was the first to propose the use of eosin in legal medicine, in a memoir written in Polish in 1883. But a long time before that this reagent had been indicated by Professor Renault in our laboratories at Lyon and in 1819 M. Clément made it the subject of his conferences in legal medicine (conferences published in 1880. J. B. Baillière and son, 1880, page 192).

I textually reproduce the procedure of M. Renault, who distinguishes himself from others as well by the use of 1:3 alcohol instead of water.

The stain will be cut in fragments of one square centimeter, each fragment placed in a watch glass and moistened with 1:3 alcohol which has no action on the spermatozoa, whereas water swells them, blanches them, and even dissolves them.

The fragments are left under a bell-jar until well saturated; one hour suffices to attain this goal.

The two faces of material are scraped with a scalpel and the scrapings placed on a glass slide, then the scraped material is dissociated on another glass slide, and the granular liquid thus liberated is mixed with that of the scraping. It is good to do the two operations just described separately and successively for all the fragments; then to individually examine the series of numbered preparations obtained.

The liquid coming from the scraping or the dissociation is finally mixed with glycerine saturated with eosin at 1 part in 200. The top slide is placed, sealed with paraffin and the preparation examined.

155 It is rare under these conditions to find completely isolated, non-fractured spermatozoa. But numerous fragments of dried sperm are encountered in the preparation; the action of the 1:3 alcohol has not sufficiently softened them to render them diffusible. These fragments are colored an intense rose by the reagent; they present breaks of conchoid appearance almost characteristic; it is these in the end which most often contain the most characteristic spermatid filaments in the state of the most complete integrity. The spermatozoa are entirely or partially engulfed in the coagulum and can easily be seen with a wide-angle objective. The head is always characteristic. An oval point of magnificent carmine red, to which is attached a filament tinted in rose, like the whole of the dried sperm, but differentiating itself by its refringence. I insist on the point that it is indispensable in preparations made as just described, to find at least one whose head is not separated from the caudal filament. Observation of isolated heads might cause confusion, and remove from the medico-legal verification all its precision.

The use of eosin is quite simply and incontestably a great progress in legal medicine as well as in general histology. Its intervention renders an enormous service in every stain, for it gives an incredible character of clarity to thousands of doubtful particles; to convince oneself, it suffices to color any type of stain with it, any mucus stain whatever, and one

will be struck by the relief taken up by epithelial cells for example.

M. Renault, in a fear a bit exaggerated, it seems to me, of destroying spermatozoa, counsels alcohol at 1:3, if this liquid is taken at a degree higher than indicated by the wise professor of Lyon, there is great risk of totally losing the stains; thus even those on glass do not give spermatozoa with strong alcohol which reduces them into fine granulations.

It is not hard to imagine that the techniques used in the laboratories of histology and especially bacteriology have been tried just about everywhere in legal medicine and at about the same time; quite particularly the double staining, so fertile in the research on microorganisms, has been tried with varied success.

I will cite especially in this context the idea of the work of a team of Ungar, in collaboration with Steilberg. These authors macerated stains for about five hours in water acidified by hydrochloric acid, one drop per cc, liquid which, according to them, conserves the spermatozoa, renders them more resistant, not without shriveling them a little. The stain is then taken with tweezers, and softly rubbed on the upper slides, which are then exposed to air until complete dessication, and finally heated three times. Ungar stains the preparations by letting them bathe in solutions covered by a bell-jar to avoid evaporation.

The stains used were: 1) eosin in saturated solution and hematoxylin (formulae of Friedlander or, better, of Boehmer). But it is necessary to leave the preparations in hematoxylin at least six days, a redhibitory defect; 2) eosin and carmine steeped in alum water (formula of Grenacher); 3) vesuvin and eosin. The solution of vesuvin contains two grams of this coloring substance, sixty-six grams of water and thirty-four grams of alcohol.

I do not insist on the inconveniences of double colorings, impractical when one finds a spermatozoon only with great difficulty, and useless when, on the contrary, there are many of them. Besides, Ungar, moreover, seems to have understood, for he proposes to simply stain the preparations with methyl green to which is added hydrochloric acid (methyl green, 0.15 to 0.30; hydrochloric acid, five to six drops; water, 100 cc.). The preparations are very beautiful if left to dry according to the procedure of Pincus.

#### Methods of the Laboratory of Legal Medicine of Lyon.

If I believed it necessary to report all the procedures which I have just presented, it stems from the conviction that in one case or another knowledge of them can render great service to an expert. Not every material lends itself equally well to the procedure of choice; *unravelling* of velvet, for example. Besides, it is not always on material that one might have to look for spermatozoa: stains on leather, on felt, on a solid body are not treated the same as those of material. The expert, then, needs to know all the procedures, and knowing which to choose as the most convenient is to his great merit. Orfila and even Donné, who in the first half of this century had the well-deserved reputation as the most able histologist

of his time, and who made himself known precisely through his studies with spermatozoa, contended that finding them in a stain was impossible. If these authors could have been acquainted with this group of procedures, which I have just briefly presented, they would certainly have changed their opinions, for they would have succeeded with one or the other of them.

When a stain is thick, all the procedures are successful: in this case we followed for a long time, and we still sometimes follow the simplest of all, that of Professor Renaut, such as we described it on page 154, but with slight modifications.

The stain, largely cut, is placed in a watch glass with some drops of water to which is added a little aqueous eosin solution. It colors intensely in carmine rose and after a little while small glaring masses, swollen by the liquid, can be perceived, more colored than the rest of the stain. These are delicately removed with a scalpel, or a cataract needle, and examined under the microscope. If necessary, the mucus is dissolved in a drop of 1/20 ammonia solution, and the action of the reagent is favored by light movements of the upper slide. The spermatozoa are very visible, and it becomes easy to preserve the preparation as material evidence. For this, the top slide is raised and the water evaporates in great part, then a very small drop of glycerinated gelatin, dissolved in a waterbath, is added, the top slide replaced and lightly pressed for more exact application against the bottom slide.<sup>12</sup>

158 But if spermatozoa are only rarely found, it is better to seal the preparation immediately with bitumen, without the risk of losing them in the manipulation which can happen in the use of gelatin.

The stain itself is then submitted to scraping with a scalpel.

But when, to the contrary, the stains are meager, imperceptible, or even difficult to find, we still use eosin to find them the most quickly, as Professor Lacassagne presented in his article *Stains* in the dictionary of Dechambre; but certain that the scraping will not give us good results, we proceed to unraveling.

Miscalculation on one hand and, on the other, the interminable hours which we are often obliged to devote to suspected stains, which we sometimes leave without positively having acquired the certitude that they could not have been formed by semen, prompted me to look for a chemical procedure, capable of eliminating suspected stains. The well-known reactions of spermine gave me no result: the most important—the spermatic odor it gives with gold chloride and magnesium—is too vague to be used in legal medicine, and besides, I once obtained it with egg white; flour paste gives it better than semen itself. One knows, moreover, that spermine has been found almost everywhere, and that this makes it lose all significance in the case in point. But it seems to me that a liquid whose physiological goal is so exalted, and which in so little volume must fulfill the highest of all functions, must be infinitely more complex than the poor analyses which have been made indicate.

It was impossible for me to believe that semen did not

contain one or many bodies, alkaloid or other, perfectly specific. Even more, it seemed natural to me to allow different principles in the semen for each species of animal, exactly as in the liver, the stomach and the humors of animals in general; chemical principles very similar, but however distinct for each species.<sup>13</sup> This admitted, my path was traced: examine under the microscope the action of all the known reagents on semen, varying the experiments in every way, such as concentration of solutions, etc. I had the satisfaction of thus finding a certain number of reactions which I will later present, only being able to present one here, which appeared to me particularly adapted to achieving the purpose I was pursuing, due to its extreme simplicity and clarity.

I had tried the reagent of Bouchardat (iodated potassium iodide) with no result, when I had the idea to try potassium triiodide, the reactions of which on the alkaloids I had formerly studied. I obtained absolutely nothing on my first attempts; I don't know why. On the recommendation of M. Bouvault, with whom I was discussing it, I prepared the reagent in a sealed tube, with the idea of assuring a more intimate union of iodine and potassium. I was not any happier, undoubtedly because, too impatient, I used the liquid while still hot.

I rationally proceeded in the opposite direction, i.e., in leaving the reagent in icewater during twenty-four hours and I obtained this time, with the semen, crystals which on first sight appeared so identical to those of hemin, that I first asked myself if, by distraction, I had not used slides used for blood research and poorly washed them!

*Preparation of the reagent.* It is most simple and I can only explain my first failures because I must have tried it by error on non-seminal stains.

Ordinary iodated potassium iodide does not give crystals with semen; quite on the contrary, it instantaneously dissolves those produced by my reagent; but as soon as the richness in iodide increases, so that the liquid contains potassium triiodide, the reaction is produced. This is not the place to discuss the state of the bodies in the solution thus obtained; I will be content to say that that which appeared the most convenient is formed simply of:

pure potassium iodide . . . . .	1.65 g
iodine (washed beforehand) . . . . .	2.54 g
distilled water . . . . .	30 g

This richness in iodine, which corresponds to KI<sub>3</sub>, is not indispensable, for perfect crystals can be obtained with a solution containing 1.65 g potassium iodide, only 1.27 g iodine, a formula corresponding to KI<sub>2</sub>. The reagent is prepared cold, very rapidly, is stable for a very long time, and is absolutely exempt from caprice. It is necessary to put it in small emery bottles whose stoppers terminate in a stem serving to obtain the drop necessary to each operation.

*Use of the reagent.* A very small fragment of stain—one thread suffices in a strict sense—is put in contact with a drop of pure water on the bottom slide; after an instant it is removed, then with the stem of the stopper or a stirring rod,

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a drop of reagent is left *beside* the droplet left on the bottom slide.

In placing the cover slip, the two liquids are mixed, in which cloudy, ochreous striations form. The crystals appear almost instantaneously; if the stain is very meager, they take a bit longer to be produced, and one can assist their appearance. When it is very hot, it is necessary to chill the reagent in ice water.

*Appearance of the crystals.* Because of the extreme sensitivity of the reaction, there is always an infinity of sizes and varied forms, but clearly characterized by a brown tint more or less dark, according to the thickness of the crystal. Addressing myself to experts, if I simply say that these crystals appear at first sight so like hemin that one can be mistaken, /161 I would be describing them better than by the longest, most minute, scholarly description. Curious thing, the form and color of hemin crystals were regarded as absolutely specific up to now; no one knew of a histochemical reaction able to cause confusion with them. And here, semen shares with blood the privilege of giving crystals so similar that description of one can serve to describe the other, without any error being able to be produced, due to the very different methods followed to attain them.

I believe then, that the fundamental type of semen crystal is identical to those of hemin: a brown on yellowish brown lamina, five or six times longer than wide, terminating at its two extremities by a hook, forming an angle variable with the direction of the crystal, a figure recalling a plank of oak flooring, called *fern leaf*.

It is the classic figure of hematin crystals, known by all: these are known to often have at their extremity a second facet, of different obliquity, making a sharp angle with the first: these I called *notched crystals*,<sup>14</sup> *cristales con escotaduras* of Carlos Demarias.<sup>15</sup> This form is also found in semen crystals, exactly as in those of hemin.

Again as hemin crystals, they fuse rather often in crosses or stars, as can be seen in photographs, and the resemblance is perfect.

But the *typical* form is not here the most common. Very often the crystals are acicular at least on one side, whereas the other terminates in two points, which gives them the aspect of a lance-head, instead of a very elongated parallelogram of primitive type.

Sometimes the crystals are united in parallel, in groups of a few, recalling *bifurcated* crystals of hemin. When the preparations are very rich, or when the crystals are nourished, /162 they become great enough to be visible to the naked eye; or, more often, thin, yellow plaques stick against them, representing parallel macles.

It was a preparation of this type that Professor Offret wished to examine; he, in our University, makes with a rare devotion his exceptional competence available to everyone, thus giving priceless services to our laboratories. Here is the note which my knowledgeable colleague wrote me:

“The substance to be examined presents itself by

microscope in *natural light* in the form of fibers of rather variable size, of a yellow brown color. The extremities form an angle of about 60° with the length of the fiber, giving an aspect of a very elongated parallelogram. The internal contour of the fibers completely disappears in this fusion and the extremities have a jagged aspect.

*Examination in parallel polarized light.* These fibers and the laminae coming from them polarize intensely in their thin parts, giving a yellow color rather like the color of the crystal in natural light. This yellow color is from a mixture of the color of the crystal itself and the tint provided by polarized light. In the thick part of the fibers, the absorption of light resulting from the intense color of the substance hinders any examination.

These fibers are rigorously extinguished following the direction of their length during their rotation between the prisms.

*Examination of converging polarized light.* This examination is very difficult to do because of the opacity of the substance in its thick parts. In using the device of Lasaulx, special for very small crystals (i.e. in removing the ocular of the microscope) the formation of two black branches of an hyperbole is noted, fusing in the center to form a cross equally diffuse, in the position corresponding to extinction in parallel polarized light. During rotation of the plate, the two branches of the hyperbole rapidly leave the field. 163

The phenomena accessible to observation are then unfortunately insufficient to determine the crystalline system. However, it was good to note the preceding results, which were indeed clear.”

M. Offret will later do new research on the crystals to determine their crystalline form which seems to belong to the clinorhombic system, even though not terminated by a facet proper.

Their size varies considerably: the largest attain a quarter of a millimeter in the preparations.

These crystals are soluble in a great quantity of cold water, very soluble in hot water. It suffices to heat the preparations to make them disappear; but they reappear on relowering the temperature. If, without sealing the preparations, one leaves them exposed to air; or, even if sealed, on leaving the reagent present, the crystals disappear bit by bit beginning at the extremities; but if a new drop of reagent is added, they reappear the following day. Ether or alcohol dissolve them instantaneously; it is the same for acids, alkali and potassium iodide. Ammonia in very weak dose leaves them intact.

If the extract of the stain on the bottom slide is completely dried and reagent added to the residue, the crystals form poorly and stay very small.

I will present elsewhere the characteristics of the principle which gives birth to the crystals, which I propose to call “virispermine”, as it appears different to me from spermins

described up to the present derived from animal testicles. Its extraction from human semen is most easy; I prepared various salts and I hope to soon be able to combine them sufficiently to establish the formula.

Up to the present, due to lack of raw materials, always starting with stains brought to me, I could only isolate very small quantities. This principle exists in rather great amount in ejaculated semen, for a large stain gave me at least ten centigrams of crystals. In treating them with magnesium, I isolated the principle itself by alcohol, unfortunately tainted by magnesium iodide.<sup>16</sup> It is soluble, acrid, and did not give me the reaction of Poehl's spermin. I will present its properties elsewhere: from the medico-legal viewpoint, it is interesting to know that it is very soluble in stains, and it resists ammoniacal putrefaction. Stains left in water in a humid place, covered in moss and emitting a fetid odor, give crystals just as well as fresh stains.

If one treats the maceration of an entire stain in a test tube with the reagent, a brick red or chocolate precipitate is produced which immediately deposits on the bottom and edges of the tube in the form of small crystals visible to the naked eye and quite remarkable: they are iridescent, russet, glistening, especially if examined in full light or by the sun.

I could not better compare them than with those old Martin polishes laden with spangled gold. In any case, a semen stain can be easily recognized without recourse to a microscope, it is so particular.

*Sensitivity of the reaction.* It is prodigious: if a small ribbon or simple thread is removed from the stain and impregnated with a small drop of water on the bottom slide, the addition of a drop of reagent gives a quantity of crystals so considerable, it is impossible to study one of them exactly, they are so numerous and entangled. Most of them, often disposed in the form of a cross, cover the field of the microscope, but on lowering the objective, one finds very small crystals in infinite number, like sand on a beach. Photograph no. 5<sup>†</sup> was obtained by treatment of a single fibril extracted from a stain: due to the diffusibility of the substance and eddies produced by application of the top slide, the crystals were dispersed throughout the preparation, but the fibril itself was all shaggy and totally obscured.

It is difficult to fix by number the limit of the sensitivity of a microchemical reaction, for this limit has to correspond exactly to the quantity necessary to obtain a characteristic crystal: thus understood, the limit of the sensitivity would be fantastic here. It is a considerable number of preparations which can be obtained with one single strip taken from a stain: if moistened, it suffices to apply it on the bottom slide and just as soon remove it; the little water left behind gives magnificent crystals, and the same strip can serve another time.

*Significance of the reaction.* To my thinking, this reaction can serve only to separate suspected stains into seminal and nonseminal: all I ask of it at the moment is to permit me to reject the latter in a few seconds and to then apply all my efforts to retrieving spermatozoa from the former. It will

certainly render service in the investigation of stains, and considerably simplify the operations of experts. But it is understandable that I cannot stop myself as of this moment from going a bit further: up to now, *I've found no product of secretion*, no liquid, nasal or vaginal mucus, urine, sweat, saliva, tears, milk, cerebral substance, hydrocele liquid, leucorrhoeal discharge, pus, etc., etc., which gave me this reaction which is so characteristic. I tried almost all the usual alkaloids, flour dough, foods, a great number of non-seminal stains, and I have never found anything comparable; the white, flowing liquid secreted by urethral glands during erection did not give me anything either. Something more curious, animal semen did not give me any crystals. I was able to try soft roe of various fish, dog semen, horse (ejaculated), rabbit (testicles), guinea pig, hare, and he-goat with no result. I do not, however, hold these results as definitive, not having repeated my attempts.

The spermin of Poehl (extract of bull testicle), that of Jacquet (guinea pig testicle) produced nothing. I believe I am then authorized to expand, until there is proof to the contrary, the significance of my reaction within the limits I will establish further on.

#### Research on Spermatozoa by Unravelling Technique.

As I have already pointed out, this procedure is incontrovertably the *procedure of choice*, to which it is necessary to have recourse in all difficult cases. It is assuredly the most rational and least brutal—consequently, the most certain. To have abandoned it in the laboratories (I have no idea why) was a serious mistake, for its failures are ascribable not to the procedure but to the clumsiness of the technician. Scraping was thought to have presented a shorter, but especially less bothersome, method to attain this end. As a matter of fact I am quite convinced that this procedure requires much more time than any other in isolating an intact spermatozoon which has escaped, as if by a miracle, the crushing to which the stain is submitted by repeated action of the scalpel. If it is necessary to intentionally fragment the spermatozoa of a stain, would it not be possible to find a more appropriate means than this violent scraping? I always have to make numerous preparations—all things considered, requiring a very long time—to find a complete spermatozoon, and this without any other benefit than the meager consolation of discerning numerous fragments which I suspect to have been the heads and tails of spermatozoa.

All this debris encourages beginners a bit, and even others, but it is agreed that they are worth nothing when it comes to conclusions, leaving the expert in annoying perplexity, if definite success does not come to crown so much work. The worst is that this scraping requires a considerable part of the stain, perhaps the entire stain, and all is irreparably lost if it has been unsuccessful! A square millimeter, a simple thread which does not alter the fabric, is largely sufficient in almost all cases, if one proceeds by unravelling.

<sup>†</sup>[Note: Photograph not reproduced in the translation].



Some preliminary considerations would not be superfluous before presenting the procedure itself.

*Instruments.* It requires the usual instruments of all operations in micrography: very fine dissociation needles, a fine forceps (like used to remove a splinter) to handle the fragments, a scalpel, small pointed scissors, watch glasses of two and a half centimeters in diameter, slides and cover slips, a good microscope, a coloring reagent, etc. I macerate the stains in small watch glasses, very rounded, thoroughly ground, all of the same size in such a way that they fit exactly on top of each other during the macerations. A certain number of these glasses, carefully labelled, are placed under an equally rounded bell-jar during the operations, where they are protected against external accidents and evaporation. Many authors have indicated the magnifications to be chosen for the observations. It is assuredly a very important, even major, point. But magnifications much too weak have almost always been recommended. Every extraction procedure isolates extremely thin fibrils, always terminating in a sort of small head, perfectly simulating spermatozoa even to the eye of a practiced technician, if observed with objectives that are too weak. The photograph<sup>†</sup> (Plate I) represents a false spermatozoon of this type which would certainly have fooled a beginner. Numerous corpuscles are always swimming in the preparations and often adhere to the extremity of a fibril. Afraid of losing this spermatozoon, sought for so long, the beginner takes great care to impart no movement to the preparation; it is enough, however, to touch the cover slip with a needle to immediately separate the fibril from what is simulating a head, and to convince him of his error. In any case, it is not the magnifications generally recommended which will help him out of this danger and even less the classic figure of spermatozoa found in all the treatises such as I have reproduced (Fig. 2) [not reproduced in the translation]. I have seen in numerous assessments stains which have nothing in common with sperm and so many pseudo spermatozoa of this type that I wonder if they have not often actually caused errors. It is strongly agreed that one can reach a conclusion in the presence of a *whole spermatozoa*, i.e., a head furnished with its tail. All that is possible in this oversimplified recommendation is confusion. This head absolutely necessitates verification of its structure and, if needed, its exact dimensions. This is possible only with high magnification.

First of all, I can think of nothing as dangerous in research of this type as continual changing of objectives and oculars; the operator's eye must absolutely familiarize itself not only with the form of the body to be looked for, but especially with the size under which the optical system shows it to him. If the objectives are often changed, this notion of size is totally ignored, whereas the habit of always keeping the same investigating objective gives the object to be found an idea of real, fixed, unchangeable size which renders confusion almost impossible. In this particular case, I use as an

<sup>†</sup> [Not reproduced in the translation.]

investigating optical system a Verick objective no. 7 and an ocular no. 3; in addition, I always pull the tube of the microscope to a reference line, marked on the length of the tube, such that with this elongation each division of a micrometric ocular corresponds to two  $\mu$ . I can adequately perceive the details of the structure of the head with this magnification due to the particular penetration of this objective, but I do not neglect in difficult cases to examine the material with an oil immersion objective. However, with most of the strong objectives of our better manufacturers, one can bypass an oil immersion system, and still be rigorous.

Today all confusion is avoided using staining reagents, useful in all procedures, but absolutely indispensable in unravelling, which often produces an infinity of fibrils similar to a caudal filament of a spermatozoon. I have already indicated (page 154 of no. 62) [this journal, vol. 11; see in these translations] the most often used. Methyl green to which is added a trace of hydrochloric acid and crocein appeared superior to all the others, but it is up to each of us to make his own choice. As for myself, I find a very great superiority in crocein which gives the details of the structure of the head so clearly without diminishing it as does methyl green in acid solution.

*Choice of strips.* All those who have done numerous assessments can remark that in the same stain certain small strips do not give spermatozoa, while others, on the contrary, give them in great numbers. Explanation of this very frequent peculiarity is easily given: when the thick and coagulated sperm of a vigorous man falls on a fabric, the fabric acts on the sperm by capillarity, drawing off the aqueous part which extends all around in a zone more or less wide; it is on the edges of this zone that concentrate the soluble principles forming the translucent border in form of a *geography map*. The spermatozoa, despite their mobility, don't appear to follow the liquid in great number; they remain fixed to the middle of the stain. It is necessary, consequently, to always cut small strips from the center of the stain, and as only a very small quantity is necessary in proper procedure—a simple thread, for example—the stains can be left with all their physiognomy intact, which is not without importance. If not, the border, saturated in the diffusible principles of sperm, is perfectly suitable for obtaining crystals by potassium triiodide.

*Procedure.* I feel obliged to give *in extenso* the procedure as first described by Roussin (*loc. cit.*, page 158), when it actually became possible, due to his iodated iodine solution.

“With very fine, very clean scissors, a small square, of a half-centimeter on a side, is cut from the center or the edge of each stain, taking the precaution not to impart any tugging to the fabric nor to cause appreciable crumpling. Two drops of distilled water are deposited on the bottom of a watch glass and taking the small suspicious fragment with the tweezers, it is gently placed on the surface of the liquid, which impregnates it bit by bit by capillarity, and completely moistens it. Experience has taught us that maceration must be prolonged for about two hours. During this time, the

watch glass is covered by only a small glass plate, to inhibit evaporation and prevent contamination by foreign bodies. Care must be taken not to engender any movement in the fabric; after an hour, it is turned over and completely immersed in the water droplets. The moistening accomplished, a magnifying glass and two fine needles jointed together are used to perform a complete unravelling in the watch glass itself, *very slowly* and meticulously of each of the threads forming the warp and web of the material. A very clean glass slide (bottom) is then chosen, on which is deposited a little of the liquid of the preceding preparation: more simply, we take all the unravelled threads with the point of the tweezers and softly touch the surface of the glass with the small humid packet. The droplet thus deposited is swiftly covered with a thin glass slide (cover slip), avoiding the capture of air bubbles as much as possible, and the finished preparation is brought to the stage of the microscope, set at an appropriate magnification.

The observation must be slow and, especially, patient: the movements engendered to the preparation to bring all of its points successively into the field of the microscope, must be methodical and extremely slow; each visible corpuscle should be studied for a long time, alternatively placed at the center and on the edge of the field; the incidence of light is frequently changed by the greater or lesser obliquity of the mirror, and the focus of each object corrected and varied by almost continual movements of lowering and raising the tube of the instrument, which is moved by a very finely threaded screw.

/254 If a certain number of cylindroconical corpuscles are discovered, and *a fortiori*, a few small isolated piriform bodies, it is almost certain, admitting that the examined stain is actually produced by semen, that an attentive and prolonged observation will bring about the discovery of a few intact zoosperms.

It happens, however, that observation of the liquid coming from the maceration gives only doubtful results: in this case, it is necessary to turn to observation of a few of the unravelled threads, and here is the best method to follow: one of the unravelled threads accompanied by a drop of liquid is deposited directly on the bottom slide, then, with a magnifying glass and two needles jointed together, unravelling is done very gently, by a movement of slow traction, to completely separate and spread out over a surface of about one square centimeter all the fibrils of hemp or cotton composing it; the preparation is covered and examined by microscope. Direct observation most often results in discovering zoosperm, if there are any; the greatest number of them are always broken; only a few can be observed intact or almost intact. The manipulation and observation is begun again on a second and a third thread, if the first is negative or insufficient. It is especially in the case of these doubtful results that the iodine solution whose formula is given above is useful. It suffices to deposit a very fine droplet on the bottom slide at the time of covering the preparation and to observe it immediately afterwards."

Here are the few modifications to which we subject the Roussin procedure at the laboratory of legal medicine:

In the middle of a stain, moistened beforehand with a drop of water and placed on the bottom slide and which gave crystals by use of a potassium triiodide solution, a small strip is cut out which must not be more than three millimeters on a side, only two if a very fine cloth (cambric) or, not wanting to alter fabric, a simple thread of three millimeters long is extracted, a rather simple operation, if after having sectioned the thread with the point of a scalpel, a thin forcep (like that for removing a splinter) is used. The strip is introduced into a very small water droplet to be moistened, and left there for about two hours. But, in general, after a much shorter time, the first attempts can begin, in delicately detaching a thread with tweezers or a needle. It is placed in a droplet of aqueous, concentrated crocein solution, where it is left for a few minutes. This solution is simply placed on a bottom slide and can be examined itself, if need be. The thread is then dissociated on a slide in a *drop of pure water* with two very fine needles. On a thread of this length, in presence of a sufficient quantity of water, the thread unravels by itself; in two or three small pulls, the filament should be resolved into elementary, well-separated fibrils, uniformly dispersed in the droplet, I should say disappeared, for one can scarcely see them. If the strip was too big, unravelling is accomplished only at the expense of considerable pulling: the fibrils mix, intertwine, cover one another, unite in groups impossible to examine, the least inconvenience of which is too big a separation between the slide and cover slip. The top does not lie flat against the bottom, and observation with an immersion objective is just about impossible. It is quite otherwise when the thread is short: the fibrils disperse themselves, separating so well they can be examined one after the other in following their widths, even under the strongest magnification. Under these conditions, it is not possible that a single spermatozoon can escape being observed, and it is astonishing to find so many in such a small fragment, when a strip of one centimeter does not give them by scraping.

In following these conditions to the letter, a thread of only one millimeter will suffice to obtain first crystals, then spermatozoa, without altering the stain itself in any appreciable manner.

*The examination.* The preparations must be carefully, methodically studied; the liquid imprisoned between the fibrils is examined first; in general, it contains very few spermatozoa, if one was too hasty in proceeding with the unravelling, but once the maceration is sufficient, they detach more and more and float into the liquid. If the procedure was performed delicately, they are ordinarily entire and intact, or if fragmented, the rupture has taken place in the length of the caudal filament almost as often as at the insertion into the head. But it is especially the fibrils which must be followed; as they are not colored, or only insignificantly. The head is very easily seen, even when adherent to their surface, and the tail appears as easily as if the spermatozoon were free. Once the maceration is sufficient, most of them, still

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held to the fibril by most of the caudal filament, present the head as entirely disengaged, but suspended by a fraction of the tail like a fruit by its stem. A whole packet of filaments emerging in bouquet from the same point is often thus found, where they are retained by a mucus mass colored in crocein. Even when the staining has not been very intense, and the spermatozoa are very weakly colored, they are easily found to the right and to the left of the fibrils, generally well spaced between each other, and in any case, easy to observe.

It is usually said they envelop the thread in great numbers like a sort of sleeve; this is an exception which must be quite rare on vegetable fibrils, because, for my part, I have never observed them, but this good fortune often happens with wool. I have seen some covering the fibril with an extraordinary regularity without causing any difficulty with the intimate observation of their structure, despite their number.

If a great number of spermatozoa are found, it is not any less necessary to verify them by an immersion objective, and all the more reason if only a few or only one are found. For this, the preparation fixed, the objective is changed and, in appropriate light, all the details of structure I previously presented are sought. If the head is disposed in profile, it is piriform, and the vesicle is not seen; but its aspect in this position appeared so characteristic that almost all authors have thus exclusively described and represented it, and rightly so.

257 It is then necessary to delicately touch the bottom slide with a needle, a slight maneuver, which most often places the head full face. If this was not fixed to the fibril by a part of the tail, and if there was fear of losing it, it would be prudent to attempt this only at a low magnification, which, giving a wider field, permits keeping the spermatozoon in sight during its flight. But a few heads conveniently positioned are almost always found, if sought with patience; the head is then of an oval configuration, the anterior part round and thin, very pale, transparent, furnished with a little vesicle—sometimes with two, small and unequal—then the transverse line, generally very clear, sometimes *obscure*, separating the posterior segment from the more colored, thicker head, less transparent, and containing, near to the insertion of the tail, a luminous, refringent point; this point is often not so visible in spermatozoa of old stains on cotton or plant material in general; finally, the small appendix, which, like an apophysis, bears the articulation with the tail. This appendix is sometimes very short in which case the facet is not less visible, or the appendix can even be nonexistent. When it extends rather far, it flares a bit to receive the articulation with the tail, whose origin is clearly indicated by the almost complete absence of color. Sometimes the head is connected to the tail, not by the juxtaposition of two facets, but by a very thin thread, the axial filament. The tail, when adherent to the head, assuredly presents the best of characteristics, and at the very most, for more security, the dimensions can be taken (plate II). But when isolated, detached, can use be made of it in legal medicine? Yes, but simply as an indication of probability, if its dimensions correspond to those of human spermatozoa, if it tapers in a regular fashion. Up to

the present, means have not been found to discern, in the case of stains, the spirals of tails, or even their division into segments. By crocein in concentrated solution, by iodide, by glacial acetic acid, the first segment can sometimes be seen, but in a fashion too inconstant for the hope of making any use of it in a difficult case.

It is necessary, for greater precision, to take particularly the diameter of the head; this operation is neither long nor difficult and gives an additional security that it would be a serious mistake to neglect; it would seem to me as necessary to indicate these measures as those of blood cells in an assessment of blood stains. If the preparations are left to dry without any other care, they become quite splendid and conserve indefinitely if sealed; the spermatozoa appear with great clarity; the vesicle does not disappear with dessication, and the transverse line seems more accentuated; the luminous point near the tail is often visible only after dessication. When a very little glycerine, about 5%, is added to the maceration water, even more splendid preparations are obtained.

#### Observations in a Few Particular Cases.

*Stains on white satin.* They are fatty, grey, translucent, and bordered by a clearer zone but not by the border of the form of a geography map. They are not very starchy, and the spermatozoa disengage with great rapidity, are splendid and very distinct. Silk fiber is not colored by crocein, so that the spermatozoa are perfectly visible on the fibril; the tails themselves stand out admirably, and are as visible as isolated ones.

*Stain on white sicilian.* The stain had penetrated through the thick fabric, appeared greyish, more milky than oily, its tint weakly yellowish, but the very fine border is luminous; a stain very starchy, stiff, rather like ribs. Under the microscope, it can be seen that this fabric is woven in cotton, and the search for spermatozoa does not present the least difficulty; a square millimeter of stain suffices.

*Stain on blue shot silk; with black tram.* The stain was very difficult to perceive; nothing in particular could be seen by transparency; a bit of stiffness marked its place; the spermatozoa stood out well on the blue fibers, but on the black fibers, it was necessary to wait till they were partly detached.

*Stain on blue cloth stippled with white thread.* (Affair of C. de Charolles). The stain was a bit whitish, and resembled that which one would have obtained by powdered rice. The fabric was very stiff. It suffices in cases of this type to dry-scrape the stain with a scalpel to loosen an abundant white dust, collected directly on a slide. A drop of water, and, after a little while, a drop of crocein, are added. The spermatozoa were quite numerous, set in a sort of viscous, layered matrix, which presented no obstacle to observation, even though colored yellow. The details of the head appear extremely clear. The stain, then treated by unravelling, give numerous spermatozoa adhering to the length of the fibrils, and which gradually detach.

*Stains on solid bodies.* These are the easiest to examine. I have already described them. Those on iron, however,

merit particular mention, because they can sometimes become very black and fail to be recognized.

In all cases of this type, it is enough to moisten the stains with a little water, and the research can proceed a few minutes later; it is sufficient to remove the shiny matter with the point of a scalpel or with a cataract needle. It is the same for stains on skin or hair agglutinated together by the semen. They are cut and macerated like those of fabric.

*Semen in the vagina, uterus, etc.* If in an autopsy, it suffices to take a little mucus from the organs, to stain it with crocein and to examine it. If the laboratory is far, the mucus must be collected on slides, or even fragments of porcelain, on which it is left to dry. But care must be taken to collect the mucus as recommended, as done in many assessments, on a cloth used to wipe the cavities! The difficulties are uselessly complicated and besides the risk of failure is high, for, in general, there are only very few spermatozoa in mucus, and it is known how little can be found in cloth stains made with very rich semen. If the operation is on someone living, a small curetage is very convenient, but it suffices, in general to wipe one's fingers on the slides after a vaginal.

*Animal semen.* The crime of bestiality, quite common in antiquity, which primitive religions even adopted in their temples, undoubtedly to limit its spread by endowing it with a sacred character, was still widespread enough in the Middle Ages to have occasioned numerous trials. These invariably ended in burning at the stake, not only of the accused but also of his victim. This horrible depravation seems to have become very rare with civilized peoples, despite what is said,<sup>17</sup> if the trials which they occasion can be a basis for judgment. They are extremely small in number, and in none of those I have reviewed have the experts used as proof the direct confirmation of the presence of spermatozoa. In the case of Pfaff, an animal hair served as proof; in that of Maschka, it was claw scratches streaking the stomach and thighs of the accused.

Apart from this crime, the expert might encounter animal semen stains made accidentally, among peasants, for example, grooms of a stud farm, etc. Soft roe of fish, which escapes so abundantly and often with force, when the fish is touched at the moment of spawning, should also be noted. Description of these spermatozoa certainly ensues from the framework I set for myself but would carry me too far from the point considering that they are very distinct from each other, even among animals which seem closely related. To cite only one example, there are among the diverse varieties of frogs absolutely dissimilar spermatozoa.

I have been able to examine quite a large number of them, but none of those I have seen can be confused with man's. Dog sperm, however, comes close, for it also has a transverse line, but no vesicle is seen in its anterior part. None of the semen I examined gave me the reaction of triiodide except man's.<sup>18</sup>

In a case of this type, it would be necessary to work by comparison, in taking sperm from the seminal vesicals of the incriminated animal.

*Confusion with other morphologic elements.* If observations are done at sufficient magnification, after staining, and the results corroborated by measurement, it is not possible to confuse foreign elements with human spermatozoa. Vaginal trichomonas, cercaria, flagellated infusoria, have been described; Frankl and Pfeiffer<sup>19</sup> have also indicated ciliated bacteria (*Trommelsch lagel bacterien*). But once again, there is nothing in these elements which would hinder a diagnosis.

**The Conclusions.** At this moment I confront a very delicate question. In the present state of science, it is by universal consensus that all authors and experts, without exception, require for the conclusion of the presence of semen that one entire, intact spermatozoon be isolated. I have said that even this minimum standard has always appeared dangerous to me in the hands of a young expert, following classical specifications to the letter, since I, for my part, have found things resembling spermatozoa under these conditions. Observations with magnifications that are too low, without preliminary staining, permit encountering elements perfectly resembling those described, especially that represented in the classical photo which is reproduced everywhere (Fig. 3). But should this reasonable specification, the prudence of which cannot be praised enough, be maintained according to the strict formula I have just written? I would like very much to say "yes," but I do not hesitate to say "no," because if I found this head so characteristic, so specific in its structure, that no possible confusion can take place; if I saw it with the exact size, form, vesicle, transverse cut, articular facet, in honor and conscience I could not declare that I have not seen the head of a human spermatozoon. There does not exist, there could not exist, a morphologic element resembling this head in all its complexity. Has there ever been any hesitation in drawing a conclusion when a blood cell has been isolated? Has there been an author who has imagined not giving a conclusion in this case? And yet, a blood cell is a simple disc with no characteristics other than its circular form, the simplest figure of all, that of spores and a multitude of other infinitely small bodies. I know well that my affirmations can be dangerous, but at this moment, I am aware only of the concern of scientific truth, and I cannot concern myself with inexperienced experts. In all questions in legal medicine, even those which seem the simplest, pulmonary docimasia, for example, the assessment is only as good as the expert himself, not the procedure. I could not truly be held responsible for the faults committed, if committed by the greatest degree of freedom I dare to give to research; for example, Mittscherlich could be accused if chemists could take the reflections of a poorly covered gas-burner for a phosphorescent glow.

But such a case will never occur. I am sure that in working correctly, whole spermatozoa will always be found, or at least even in the most complicated of cases, a number of heads such that there can be no place for doubt. Moreover, tails will be found, which, it is true, have no major characteristic, but their weak staining, their diameter regularly taper-

ing into an extremely fine point, and especially, their total length, exactly measured, are certainly not to be neglected and will corroborate the convictions of the expert.

And if this extraordinary case occurs, the reaction of triiodide fortunately provides additional means to eradicate all doubt.

I have to examine the case where only the crystals would be produced, for unknown reasons. Since the works of Rattier, Bayard and Devergie, it is unanimous to refuse to give any value whatever to chemical reactions in the investigation of seminal stains. Tardieu, Casper, Von Hoffman, Taylor, Tourdes, Brouardel, Vibert, G. Pouchet, Lacassagne, Coutagne, Boutmy, Maschka, briefly, all the authoritative masters on the question, are in absolute agreement.<sup>20</sup> At the very most, there was found, formerly an observer who noted the presence of an albumin coagulable by heat—it does not exist in semen—permitting differentiation of this from other mucus; but this has been forgotten a long time. Outside of this, everything has been rejected, and with good reason, since only vague reactions, in no way justifying the pretensions of their authors, were proposed. But it is no longer a matter of an albumin or a mucus that one must try to distinguish by feeble nuance, but a new characteristic, specific principle, secreted exclusively by the testicle, a principle I encountered nowhere else, not even in animal semen, [which I discovered]; and, until there is proof to the contrary, its significance cannot be refused. No substance is more clearly characterized by its chemical properties, and such a delicate reaction, and none is easier to find. I hope, then, that the excommunication, legitimately levelled against chemical reactions applied to the investigation of semen, is not addressed to mine.

However, I know that a sort of sanction, of consecration, if you will, that only time, and the examination of numerous humors, can give, is necessary to a reaction of this importance. In awaiting it, here is the way I would conclude in difficult cases:

1) If I obtain crystals without any spermatozoan heads, I would say I am *probably* dealing with semen, and I would emphasize in my conclusions: "considering that no known humor other than human semen has up to the present given these crystals."

2) If I obtain crystals and, at the same time, only the heads of spermatozoa *perfectly characterized*, I would clearly conclude in the affirmative.

3) If I obtain only debris of spermatozoa, even with perfectly characterized heads, but without my reaction, I would still remain doubtful, considering that there can be found in animal semen spermatozoa resembling that of man, giving rise to an error, *although I don't believe it* for my part.

If I have not resolved in this work all the questions I proposed, I have no less the conviction of having rendered real service to experts in bringing them, by my triiodide reaction, a method as simple as it is easy for differentiating suspected stains in a few seconds; by the *modus faciendi* which I indicated for unraveling, I gave them the means for

looking at large numbers of preparations, and finally, with crocein, I gave them the possibility of confidently concluding the presence of semen, even when an entire spermatozoa has not been isolated.

I am certain they can finish in less than two hours assessments which, before would have required perhaps many weeks, and that they can attain an absolute certainty in numerous cases where incontestably firm conclusions had been impossible.

**Additional note:** On April 6, the centenarian P.V. . . . died at the home for the aged at Guillotiere, at Lyon. Born July 20, 1794, he was, consequently, 102 years old. A police record mentioning the curious tatoos of P.V. . . . leaves no doubt as to the exact age of the old fellow, nor to his identity, a rather rare circumstance, considering it is believed that, most often, centenarians simply use the papers of their father. The autopsy was done at the laboratory of legal medicine by Professor Tripier, Doctor Pavot and myself. The seminal vesicles contained a reddish, rather thick liquid, containing spermatozoa, giving with crocein all the characteristics I have stated in this memoir. In addition, we found fatty globules and a large number of rose, blackberry-like, spiny corpuscles of various sizes in this liquid. After desiccation, this liquid was revived with a few drops of water to separate the fat, and it then gave crystals with triiodide.

It remains definitive, then, a fact surrounded with all desirable guarantees and duly confirmed, that man's semen contains perfect spermatozoa up to extreme old age.

A. F.

#### References and Notes

1. Pope from 1198 to 1216
2. Lacassagne: *Précis de médecine judiciaire*, p. 104
3. It was believed that conception could not have taken place if the woman had not consented to the act; from the fact that she was pregnant, it was agreed that she was voluntarily taken!
4. "Jurisconsults have judged virginity during fourteen hundred years, as they judged sorcery, and so many other cases, without understanding anything."
5. These globules, sometimes seen immobile and sometimes endowed with motion, are pointed out in almost all the first microscopical investigations of stains.
6. I find this name written Koblauch, just as often as Koblank
7. With our modern microscopes, it can be seen in its entirety
8. *Annales d'hygiène et de médecine légale*, vol. 27, 1867, p. 155
9. *Lehrbuch des Gerichtl. Medicine—Vierteljahrschr. f. gerichtl. Medicine* 1887, p. 318
10. Clément had already made the same reproach (*Conférence de médecine légale*, 1880)
11. These granulations exist in almost all stains; they are spores
12. Glycerinated gelatin of Kaiser: one part by weight of purest French gelatin in six parts of distilled water is allowed to soften for about two hours. Seven parts of chemically pure glycerin is then added and one gram of concentrated phenol is added to 100 grams of mixture. This is heated for 10–15 minutes with continual shaking, until the flakes formed by the addition of acetic acid have disappeared. This is filtered while hot on a very fine glass wool, still humid from washing by distilled water.
13. The biliary acids of man are different from those of pig, which are not identical to those of goose.
14. Florence. *Des taches de sang en méd. judiciaire*, p. 79, 1885

15. *Las Manchas de Sangre*, Montivideo, 1894
16. This is why silver oxide must be employed, giving a pure crystallized product
17. Martineau, Tardieu, Schauenstein
18. Spontaneous crystallization observed in dried semen on glass slides is different, and diverse varieties of crystals can be found even in a single semen: that of horse contains at least two, if not three, which perhaps corresponds to as many spermins.
19. Cited by von Hoffmann
20. Bayard: chemical analysis is insufficient to resolve it . . . but microscopical examination permits better specification of the observed facts (*Med. leg.*, p. 274).

Roussin (*Loc. cit.* p. 148): There are so many chemical reactions applied to the determination of semen; none of them characterize this secretion. The impotence of chemical means today has been demonstrated so well, and is so universally recognized, that it appears useless for us to insist on it.

Gorup Bezanès (*Traité d'analyse zoochimique*, 1875, trad. de L. Gautier, p. 422). But all these reactions are not sensitive enough to permit drawing a certain conclusion, and stains produced by different mucus give rise to similar reactions. Only confirmation of the presence of spermatozoa by microscopical examination can furnish positive indications.

Bouisin Briand and Chaudé: As for chemical reactions indicated by

many authors, they have no value next to the preceding, and experience proves that almost all stains formed by mucus also show them.

Brouardel and Boutmy (*Annales*, 1880, p. 225): In effect, the chemical reactions which can serve to characterize the liquids of the organism are very restricted in number, and are generally limited to either coagulation by heat, or by a few reagents such as nitric acid, mercuric chloride, phenol or wine alcohol, or to various colorations which certain substances cause to appear in the material being examined.

As a result, the chemical reactions we have just presented, applied to the study of the organism, indicate the class of the material rather than its particular identity.

The presence of the anatomic element, which is always unique, by contrast, makes confusion impossible, and the expert is always able to give an opinion with every assurance, etc.

Vogel (*Vierteljahrschrift*, N.F. XXXVI, p. 160, 1882): Under these conditions, it is certain—since there are no characteristic reactions—that microscopical investigation of the morphological elements of semen stands alone, and that spermatozoa always remains the only certain sign.

Taylor (*Méd. lég.*, trad. de H. Coutagne, 823): There are no chemical reactions on which one can count with certainty for discovering seminal stains

*Real Encyclopedie*, v. IX, p. 31: Only the finding of spermatozoa by the microscope is a certain sign that one is dealing with a seminal stain.

## A New Microchemical Reaction of the Sperma and its Application in Medico-legal Investigations\*

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7383 The search after a sure method to recognize stains caused by semen has been on account of its medico-legal importance, one of the most interesting and the most debated questions to which during the last years men interested in medico-legal questions have given their attention.

The surest and easiest method, which deals with the recognition of the spermatozoa, very often meets in practice with insurmountable difficulties, without even mentioning those rare, but not impossible cases, in which there is azoospermia (sperma without spermatozoa) and in which this method naturally loses all value.

Among the methods which have been proposed for the purpose of demonstrating in the best way possible the spermatozoa, it will be sufficient to mention those of Bayard,<sup>1</sup> of Schmidt,<sup>2</sup> of Koblanck,<sup>3</sup> modified and perfected by Pincus,<sup>4</sup> by Hofmann,<sup>5</sup> by Longuet,<sup>6</sup> and the method of Unger<sup>7</sup> and Steilberger,<sup>8</sup> and these methods are surely the most simple and the most rapid, and are superior to all the other methods by the safety of the results.

7384 But the spermatozoon, like other cellular structures, may through different agents, some chemical and others physical, undergo such changes, that it becomes hardly or not at all recognizable, thus making it impossible for the expert to make a decision with the certainty which the law demands. Given this insufficiency of histological methods, the idea naturally occurs to one to find means which are more appropriate and which have a larger field of application. Therefore, we have turned to chemistry and worked on the proposition that the sperma must contain certain special substances which must be capable of giving constant and characteristic reactions.

The first attempt in this direction was made by Orfila,<sup>9</sup> to whom it seemed that dilute nitric acid, although it caused a yellow color in organic liquids which contained albumin, did not change the color of the sperma, which contained none. The reaction of Orfila was not confirmed, and the opinion, that the sperma contained no albuminoid substances was proven to be erroneous by Posner,<sup>10</sup> who has given to the

study of the sperma various valuable contributions, and more recently by Slowtsoff.<sup>11</sup>

To this is to be added that nitric acid, as proven by the research of Filomusi-Guelfi,<sup>12</sup> destroys the spermatozoa, thus interfering with histological tests. After the failure of this test, many years passed before this research was taken up again, and it was only in the year 1895 that Florence,<sup>13</sup> in a publication which made a great deal of commotion in the camp of legal medicine, showed a new micro-chemical method for the recognition of seminal stains, strengthening this with numerous comparative tests, none of which showed the same reaction.

The reagent which he mentioned is a concentrated solution of iodine in iodide of potash, in a proportion which makes a highly concentrated iodine salt; the triiodurate of this reagent, on contact with the sperma, causes a formation of strong yellow crystals. The reaction was constant, simple and very sensitive and could be applied with equal ease to dry as to liquid sperma, to fresh sperma as well as to stale sperma, whether in good or in bad state of preservation.

Florence considered the reaction as the product of the action of the triiodure on an alkaloidal substance contained in the sperma, the virispermine, a substance not only specific to the sperma but characteristic of human sperma.

The problem seemed solved in a most brilliant and decisive way, and those interested in legal medicine hurried to repeat the tests made by Florence for the purpose of enlarging and deepening the tests and experiments with sperma.

In a short time the following works saw the light of day: The one of Lecco,<sup>14</sup> of Richter,<sup>15</sup> of Gumprecht,<sup>16</sup> of Struve,<sup>17</sup> of Tamassia,<sup>18</sup> of Mattei,<sup>19</sup> of Tolsky,<sup>20</sup> of Davidoff,<sup>21</sup> of Caneva,<sup>22</sup> of Witalinsky and Horszkiewicz,<sup>23</sup> of Bocarius,<sup>24</sup> of Perrando,<sup>25</sup> of Ponzio,<sup>26</sup> of Dwornitschenko,<sup>27</sup> of Centner and Ramsaizeff,<sup>28</sup> of Mary<sup>29</sup> of Korsunsky,<sup>30</sup> of De Crecchio,<sup>31</sup> of Johnston and Witney,<sup>32</sup> of Cruz,<sup>33</sup> of Grigorieff,<sup>34</sup> of Gutowsky,<sup>35</sup> of Okamoto,<sup>36</sup> of Goldschmidt,<sup>37</sup> of Beumer,<sup>38</sup> and of Kippenberger,<sup>39</sup> a mass of studies sufficiently vast, which in short time has dissected this grave and delicate argument.

The evidence of many of the works just cited points to the result that the claim of Florence is not sufficiently backed up by fact, and that not only the sperma of many animals, but also many different organic liquids, both physiological and pathological, show the same reaction very plainly.

And in consequence of this general opinion, not con-

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tradicted by anybody but Johnston and Witney, Strassmann<sup>40</sup> states that the reaction of Florence is not only not specific of the human sperma, but even that it can be absent in the presence of human sperma, when this is mixed with blood.

Other cases in which the reaction gives negative results are those in which the liquid sperma is in a state of advanced putrefaction.

These results have taken from the reaction of Florence the value claimed for it by its author and have limited the same to that of a preliminary test.

386 A negative result, according to Strassmann, does not authorize one to claim that seminal stains are absent, while Goldschmidt and Hager-Mez,<sup>41</sup> claim that the absence of this reaction is a sure proof of the absence of sperma.

In conclusion, the reaction of Florence, which according to the idea of the discoverer, was decisive proof of the indication of seminal stains, has an importance much inferior to that of the histological proof, and cannot alone solve the question.

Having thus shown the present state of the question, I will without further introduction show the result of my studies, which are the fruit of many tests and experiments, made in the course of several years. I have made use of a great deal of human sperma, taken in all cases, except two, from healthy and not from sterile individuals. I have made the tests with sperma not over a few minutes old, as well as with such as had dried on cloth, also with sperma kept in glass vessels which were sealed and with other in a state of putrefaction. The result has always been the same, and causes me to claim that this reaction is safe and belongs particularly to seminal liquid.

The reaction is made in the following way: Put a drop of the sperma on a covered glass. Add to this a small quantity of a watery saturated solution of picric acid, a quantity not more than one-half of the liquid to be examined. After a few seconds the spermatic liquid will become turbid, through the formation of a precipitate, which, limited at first to the point of contact of the two liquids, little by little mixes itself with the two, spreads over the whole drop, which acquires a yellow color and a turbid appearance.

After a few minutes, between two and five generally, the reaction can be said to be complete, and after having placed on top of the liquid a cover glass, one proceeds with the microscopical examination.

If instead of liquid sperma one has dried sperma, all one has to do is to soften it with a little water, the same as one would do, if the sperma were dried on cloth. Then one adds to the water liquid, which should not be diluted with the picric acid solution.

387 If the liquid was very diluted, the formation of the precipitate would be slower; also, if there was an excess of picric acid, or the picric acid solution was too weak, the reaction would not be so clear, and the precipitate might assume a granular form, causing it to lose partly or entirely its demonstrative value.

The picric acid, besides being used in aqueous solution, can also be used in a saturated solution of absolute alcohol; in fact, in certain cases, as for example in cases in which one has to deal with putrefied sperma, the latter gives better results.

But in the case in which one takes the alcoholic solution, one must, to avoid the mixing, which would follow the contact of the two liquids, and which would cause a too rapid evaporation of this hydro-alcoholic mixture, reduce the quantity of liquid to be examined very much, in fact use less than a pin's head, and also reduce very much the quantity of the picric acid to be used.

Thus I would advise to begin the tests with a very small quantity of picric acid, which is to be added to the solution to be tested by means of a platinum needle and then after a few minutes one can add a little more, for the purpose of obtaining a diffused and noticeable turbidity.

Further, it is sufficient to make two or three preparations with liquid sperma or with an aqueous extract of a seminal stain, for the purpose of ascertaining the proper proportions of the two liquids, so as to be sure to obtain perfect preparations.

One thing more in regard to the cover glass. This should be neither too small, so that the liquid does not run over the sides, nor too large. Thus, by placing the cover glass carefully on the drop, which has first been deposited on the slide, the crystals will remain together and are not squeezed out of shape.

The microscopical examination, which is made under an enlargement of from 400 to 600 diameters, shows that the precipitate resulting from the reaction consists of small crystals, yellow and strongly refractive. One look at the attached plate, which reproduces faithfully these forms, designed by means of the camera lucida, will teach as much as the most minute and accurate description.

Without entering for the moment into the question of the shape and of the crystallographic points of these crystals, which would be a rather complex and difficult question, I will only say, that these crystals, which are four or five times longer than they are wide, are very thin, appear like needles with rhombic circumference, and traversed longitudinally by a refringent line, which has the appearance of an edge. The obtuse angles seem always to be worn off and rounded, and in not well shaped crystals even the other angles have often the same appearance. In the last case the crystal appears to have the shape of an ovoid body, sometimes more, sometimes less elongated, which in extreme cases assumes the shape of a round disk. From this shape to the perfect rhombical shape we find a whole line of intermediate forms, which represent different stages of development, and which are very numerous and characteristic.

Besides isolated crystals, which prevail in number, one finds also twin crystals, others hanging together in the shape of a cross and others again in clusters. The table shows various forms of collections, as well pure as imperfect formations, in which only polarized light can reveal an entirely



crystalline structure.

I have already mentioned that these crystals are very refringent. Now I will state that they are also very much birefringent. The examination with polarized light gives the proof of this, as also it permits various vivid colors of interference to be observed with an enlargement of 60 diameters.

The exact measurement of the angles was not possible to me, in view of the smallness of the crystals and their imperfect formation, and the values vary from eight to ten degrees. The size of the crystals varies from 5 microns to 20 and more microns, although I do not claim that there cannot be smaller or in good conditions larger ones. The medium size, however, which responds to the majority of forms seen, and which are produced under ordinary conditions, varies from ten to fifteen microns.

The reaction can be noticed as well in acid as in alkaline media, as long as neither acidity nor alkalinity surpass certain limits. So, instead of the picric acid solution the picro citric acid solution of Esbach can be used or a solution of picrate of ammonia.

But I prefer the picric acid solution to all others.

The reaction is very sensitive and sufficient to detect the very smallest quantity of sperma. It can be produced as well in fresh sperma as in dried sperma or in sperma which is in a state of putrefaction. And, in respect to putrefied sperma, the reaction is much more sensitive than that of Florence, so much so in fact, that I have received positive results in cases in which the reaction of Florence has failed. The presence of blood, as long as it is not in excessive quantities, does not disturb the reaction. Neither does the action of heat, within certain limits, make any difference.

At 10 degrees the liquid sperma as well as the dessicated sperma give a positive reaction, even after several hours.

At a high temperature the effect with the two kinds of sperma (liquid or dried) is different. Liquid sperma, which is kept at a temperature of 110 degrees for an hour does no more give the reaction. At 132 degrees ten minutes is sufficient to give a negative result; at 143 to 146 degrees five minutes. Sperma, however, which is dried in the clothing, is capable to stand a heat of 150 degrees for an hour, without interfering with the reaction. But carried to higher temperature the reaction shows less well, and carried to 200 degrees a few minutes suffice to cause the reaction to fail. I have stated that a heating to one hundred degrees does not harm, but I could even say that it helps the reaction, in the sense that the crystals which are formed are more beautiful and better formed.

The best preparations I have obtained from stains on linen, which had been exposed for an hour to a temperature of 130 in a dry oven.

In regards to the age I will submit the following data: Putrefied sperma, kept in well stoppered bottle gives a positive result after eight months. Dried sperma on linen even after three years. This I have verified on sperma kindly furnished to me through the courtesy of the celebrated Prof. Corrado, director of the Medico-Legal Institute of this

University, to whom I owe for this, as well as other assistance, the most heartfelt gratitude.

To make a durable preparation the preparation must be dried, then quickly washed in water, dried in blotting paper, again washed with alcohol, bathed in xilol and lastly mounted in Balsam.

The question now arises, whether these crystals represent a definite combination between some organic principle of the sperma and picric acid, or result from a union of the picric acid with the inorganic bases of the sperma, if not only with substances due to the decomposition of the sperma? Which means to say, that either this reaction has a useful value in practice, or owing to a substance which does not belong exclusively to the sperma, has no value whatsoever.

Yet the question must be answered, what is this substance, and whence does it come?

I might multiply these questions, but for the moment I will limit myself to those which form the kernel of the question and which are the most obvious. So let us commence with the most simple hypothesis, that we have to deal simply with picric acid crystals. However, we must observe that the formation of picric acid crystals in consequence of the mixing of a solution of this acid with an aqueous solution which is rich in bases with which it could easily enter into combination is not a fact which can be brought in harmony with the laws of chemistry. And even if the acid had been added in excess, even if only a part remain free, this would remain in solution, and could not become deposited except in consequence of the concentration of the liquid beyond the limit of saturation.

In our case, however, the reaction appears at once and the crystals form before such an evaporation could take place. Besides there exists a noticeable difference between picric acid crystals and those which form through the action of the acid on the sperma.

The acid crystals are formed of rhombic prisms, which show an entirely different formation under the microscope. Besides the different shape of the crystals, the difference in color must be noticed.

Picric acid crystals show under the microscope a very slight yellow color, hardly visible with strong enlargement, while our specific crystals, even when observed with an enlargement of 600 diameters and more, show always a decided yellow.

Lastly, I wish to observe that the best solvents of picric acid are Benzol, which at ordinary temperature dissolves from 8 to 10 percent (Fritzsche), and in Xilol, which dissolves even 14 percent.<sup>42</sup> Both Xilol and Benzol, however, are the worst solvents of the specific crystals.

A second objection might be that we have to deal with an alkaline picrate and more especially with picrate of potassium, which among all is the least soluble. One need not think of either the picrate of sodium or of calcium, because they are very soluble in water, the first in from 10 to 14 parts, at 15 degrees, the second in a like measure at a temperature of 20 degrees. One may also exclude the possibility that one

has to deal with picrate of ammonia, as ammonia, as is proven by all the analyses, both of Liebermann,<sup>43</sup> as well as of Slowtsoff, is not a usual part of sperma, and is not formed in the same except as a result of decomposition. And the sperma reacts to the picric acid within a few seconds after ejaculation, and besides, the reagent of Nessler, which is, as everyone knows, very sensitive to ammonium salts, does not show the slightest trace of the same.

The picrate of potassium again is a salt which is nearly insoluble in cold water, one part, according to Post and Mehrtens soluble in 228.17 parts at 15 degrees. My crystals however are easily soluble in water.

It is not easy to determine what the substance may be which causes the sperma to react, as it does, to the picric acid. The fact that the reaction fails, after the sperma has been subjected to 200 degrees, justifies the suspicion that one has to deal with an organic substance. A fact, which must be kept in mind, is that the reaction can be obtained as well in acid as in alkaline or neutral solution, analogous to the one which Popoff<sup>46</sup> has found in the alkaloids.

In regards to the specific character of the reaction I wish to state, that no matter how many substances I have examined, including vaginal mucus, nasal mucus, sputum and so forth, none has given the same result, none have formed the same crystals as the seminal liquid.

392 The only substance, which when treated with picric acid gives similar, however not like results, is Poehl's spermin. This latter, however, heated for half an hour to one hundred degrees loses the power of forming a crystalline precipitate with the picric acid, forming instead a precipitate consisting of granules which appear oily and which possess not the slightest birefringence.

Lastly, I wish to state, that the seminal vesicles after death fail to show the reaction. Thus without stating what causes the reaction, I will only say that it is due to an organic substance, which has nothing to do with the one which produces the reaction of Florence, and which is contained also in the sperma of sterile individuals.

This reaction has, besides the advantage, that it can give durable preparations, also that advantage over the reaction of Florence, that it is absent in the presence of other substances than sperma, and which react under Florence's reagent like sperma.

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## On Forensic Identification of Semen and Semen Stains\*

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2131 Among legal medicine's effective investigations, the identification of semen stains occupies a quantitatively dominant role, particularly on clothing. The methods that have been used until now are either chemical or physical reactions or histologic proof of spermatozoa.

The chemical reactions that are most often used, stem from the time around the turn of the century, when knowledge of the characteristic components and complete chemical content of semen was scanty.

The classic Florence test is carried out by having a well-soaked extract of stains brought into contact with a strong iodine-potassium iodide solution on a microscope slide. In this way brown rhombic crystals can appear that presumably consist of iodine. The reaction is caused by the presence of choline, that can be thought to arise as a degradation product of lecithin that is found in semen in considerable amounts. Before the Florence test yields positive results, some decomposition of semen occurs, and thus the test often fails where it concerns itself with fresh semen stains. Since lecithin also can occur in other biologic material the reaction is not specific. In this connection it is especially unfortunate that vaginal secretions now and then yield positive reactions.

Another chemical test that has been put to use is the formation of calcium sulfate crystals by addition of sulfuric acid to a stain extract. Now the calcium content in the semen is about double the quantity that is in blood; thus, one may already reject the test for this lessens its diagnostic worth.

Semen's characteristic smell that by itself can be a valuable guide in the search for stains, is due to a base, spermin, that has occurred in considerably large amounts only in other secretions and excretions.

In many tissues, the liver and pancreas, for example, spermin is found in a concentration approximately one eighth the concentration of normal semen. In such tissues spermin is hardly extracted by means of simple treatment with salt water.

Many methods for identification of semen stains consist of the development of saturated solution binding to spermin with different acids. In this manner *Barberio* (1911) used

picric acid as the crystallizing agent, but experience has proven that the reaction is most doubtful. In a comparative investigation of *Ziemke*, the Florence reaction was found positive in 70%, and *Barberio's* reaction positive in 26%, of the cases investigated.

Great interest is attached to *Puranen's* crystallizing method (1936). He used naphthol yellow S (sodium-2, 4-dinitro-1-naphtholsulfonate; sodium flavianate) as the reagent. In the presence of spermin, characteristic crooked cross shapes are formed with short cross beams providing crystals of spermine flavianate. This test is undoubtedly the best of the existing chemical methods, but also it suffers from serious deficiencies. Many commonly occurring textiles, certain colors of artificial silk, leather or natural silk materials, for example, cause the reaction to be lacking, presumably because of strong absorption. Often one can get a positive reaction by itself on these textiles by using the sufficiently small extraction volume. *B. Jonsson* has observed spermatozoa in a good number of instances, where *Puranen's* reaction appeared negative.

The possibility of getting a positive reaction although the semen is not present is certainly possible, as the aforementioned spermin content in other tissues and secretions and in different biologic materials can by no means be ignored.

A simple physical method for identification of stains that involves a great deal of extensive diffusion is the investigation in ultraviolet light. Semen can contain substances that fluoresce strongly under light with wave lengths from 4200 to 4900 Å. However, the reaction is by no means specific because firstly, semen does not always contain the fluorescent substance; secondly, fluorescence is seen in practically all the secretions or excretions that can be counted among some interfering sources (vaginal secretions, nasal secretions, urine, feces, etc.); and, thirdly, the fluorescence disappears with the mixture of, for example, blood. However the investigation with ultraviolet light has meaning in that it is a valuable means for the discovery of the suspected stains.

The purpose of the histologic methods is of course to identify spermatozoa. One can either identify these cells on the isolated stained fibers of the fabric, or after a thorough extraction one can centrifuge the extract and attempt to observe the sperm in the deposit. Unstained preparations are used at some institutions; at others, preparations are stained

\* Translation of: "Om forensisk paavisning of sperma og spermapletter." in *Nordisk Medicin* 28 (42): 2131-2132 (1945).

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132 according to more or less specific methods. Among the staining methods, *Baecchi's* is reputed to be the most widespread. The stain consists of acid fuchsin and methylene blue in 1% hydrochloric acid, and reacts with the successful result that the main body of the sperm is colored red, while the tail remains blue. The methods have proven themselves satisfactory through investigations over many years. Here at home, the *Ellermann* staining method is also used sometimes.

Microscopical identification of spermatozoa does not cause any difficulty in general. When one takes into consideration that a normal ejaculation consists of about 100-200 million sperm per ml one can understand that it is usually no problem to detect the outermost characteristic cells in the microscope. Nevertheless, the method is not ideal for the following reasons: 1) the composition of the ejaculate is not constant during the ejaculation; the first part contains almost exclusively of prostate secretion, while the spermatozoa first appear only in the later phases of the ejaculation. Because the forensically most important stains are often not formed of homogeneous semen, one can get stains for the investigation that do not contain spermatozoa, although they are made up of parts of a standard ejaculation. 2) Oligospermia and total aspermia are not unusual conditions and can yield semen stains that cannot be visualized; 3) Sterilization by vasectomy is now undertaken so frequently that especially among sex offenders one can expect to find such instances in forensic practice.

For this reason it would be valuable to have a specific method for the identification of semen that is independent of the presence of sperm. If one pays attention to the recent chemical and biochemical investigations on the composition of semen, one will find that most substances in semen are found in an increasingly greater concentration than in the body's remaining tissues and secretions. Acid-soluble phosphate is found in a quantity of over 100 m% (m% = 1/1,000% = mg%), while the concentration in, for example, blood is around 30 m%. Now the phosphate is such a diffuse material that it will hardly be worthwhile to try to use it for evidence of semen, particularly considering that urine can contain significant quantities of phosphate itself.

Ascorbic acid is certain to occur in semen in concentrations about 10 times as high as in blood plasma; but this material is, of course, so unstable and generally also so widely distributed that it cannot be considered either.

In 1936 *Schersten* proved that semen contains a large quantity of citric acid, about 0.5%. When one has sensitive, even if somewhat difficult, methods for determination of citric acid, there should be a possibility here for working out a procedure for specific evidence of semen, yet certainly only on the assumption that other stain-forming materials do not contain citric acid in quantities that can be compared with quantities in semen. Investigations on the proportion of citric acids are in process at the Institute.

A possibility that finally appears more promising is using the phosphatase content for the diagnosis of semen and semen stains. In the prostate secretion, there is a phosphatase with an acid pH optimum in colossal quantities. The enzyme was first found by *Kutscher & Wolberg* in 1935 and has been later investigated biochemically by *Kutscher* and collaborators, and from a clinical point of view in particular by American writers. *Gutman & Gutman* find 2,000-3,000 phosphatase units per ml semen; of that, the lowest value among 43 ejaculates is 540 units per ml. 24 aspermic individuals, with the exception of one, had values inside the normal range.

Even though the enzyme in semen is found in a large concentration in some other biologic material, and enzymes generally endure drying out well, there were reasons to expect that this method could lead to very specific evidence of semen stains independent of the presence of sperm cells, possibly even to a practical, valuable forensic method. When, therefore, it was time for the present director of the Institute in 1943 to propose the university's prize task in theoretical medicine, the author suggested to Prof. *Knud Sand* that the task be an experimental investigation on the use of phosphatase determination for identification of semen stains. The current examination papers, of which three have been rewarded with the University's gold medal, have shown that our expectations were justified. The three authors intend to publish the results of their investigations in the near future.



Dr. Paul Uhlenhuth 1870-1957  
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