THE EXAMINATION OF SEMINAL STAINS.

An investigation into the different methods advocated.

by

Douglas.J.A.Kerr, M.B.,Ch.B.,D.P.H.

1920

Gunning Victoria Jubilee Prize in Forensic Medicine 1923

THE EXAMINATION OF SEMINAL STAINS.

The tests for seminal stains are found to fall into three groups, (1) The rough preliminary tests of Florence and Barbario, (2) The actual finding of the spermatozoa, under the microscope, and (3) The biological test for semen.

Farnum[1], Uhlenhuth[2], H. Pfeiffer, more recently Dervieux, and last year Hekteod[3], have applied the biological test for blood to the detection of semen. They have induced the formation of precipitins, in rabbits blood, for human semen and human serum, by elective abso--rption the precipitins for human serum have been removed and a serum obtained which reacts to human semen alone, blood serum, pus, ascitic fluid, etc and the semen of the bull, dog, goat, guinea pig, and rabbit giving negative results.

This method whilst being of scientific interest is unneces--sarily elaborate, as if proper methods are used the spermatozoa can usually be demonstrated with the microscope. In the series of stains examined spermatozoa were demonstrated in every case, although some of the stains had received vigorous washing and others were as old as twentytwo years.

It is to the first two tests then that attention will be paid in this investigation.

When the leading Text-books on Forensic Medicine are consulted, each one is found to give a different method for the detection of sperma--tozoa. The following examples illustrate this very well.

TAYLOR[8], places the fabric to be examined in .9% sodium chloride and 10% glycerine for one hour, he then removes the fabric and presses it on to a slide; he advises that it should be moved as little as possible

E. UNGAR[4] moistens with dilute hydrochloric acid (1 drop in 1½ ounces of water) leaving only one end of the fabric in the fluid, he leaves it to soak for five hours and then touches the slide with it.

DIXON MANN[9] mixes methyl green with his solvent which is a modified form of Ungar's method.

GUY and FERRIER[10] place the fragment in distilled water for fifteen minutes and then examine it.

PETERSON and HAINES[11] say, soak for at least two hours in water, tease with fine needles and examine, stain if necessary.

DRAPER[12] of Harvard moistens with water, teases and stains with methyl green.

GLAISTER[13] macerates in .3% acetic acid for two hours and then touches slides with the fabric, he then stains the slides with gentian violet.

As is seen above the choice of methods is very variable, so one of the first points to be investigated was the comparative values of the

different solvents and methods advocated. In order to ensure as far as possible, the same conditions for each slide, artificial illum--ination from a carbon filament lamp was used throughout.

POINTS INVESTIGATED.

Macerating fluids.

The following solvents appear to be thost chiefly recommended:-

Water.

Glycerine and Water (2pts glycerine to 16 pts water).

Glycerine(10%) and Sodium Chloride(.9%).

Sodium Chloride (.9%).

Acetic acid (.3%).

Hydrochloric acid (1 in 700--Ungar).

Erythrosine(2%) in strong Ammonia.

Glycerine Pepsin B.P. (Richter).

Roussin's Fluid (sulphuric acid 1, glycerine 3, water till S.G.is 1028).

Pacini's Fluid (NaCl 4gms, glycerine 26, mercuric chloride 2gms, water226c

The last three fluids were originally recommended for the detection of blood corpuscles in blood stains; it was thought that they might be of use in connection with seminal stains, as is shown later, the results with the first two make the use of other macerating fluids appear a waste of time, especially if the stain is old.

A series of stains were taken, treated with each of these fluids and examined with a view to determining the ease and quickness with which spermatozoa could be detected.

As some reagents are stated to distort the spermatozoa beyond reco--gnition,or to cause them to disintegrate,those of the above fluids which were considered to be of use were allowed to act on smears from the seminal vesicles,which consisted entirely of spermatozoa, and the results noted.

Staining.

The difference of opinion as to the best methods to use is not confined to macerating fluids,but is also found in regard to stains,if any,to be employed.

The following stains were investigated as to their staining effect onspermatozoa and their action on certain of the above macerating Fluids.

Leishman.

Zieehl Neilsen.

Gram.

Gentian Violet.

Picro-Carmine.

Iodine in Potassium Iodide.

Erythrosine (aqu: sol:).

Erythrosine in Ammonia (Cordonnier and Muller)[6].

Eosin and Methyl Green.

Carbol Fuchsin.

Dried watery Preparations.

Washing.

The effect of washing in hot water and in hot soap and water was tried.

Chemical Tests.

The two methods commonly recognised, namely Florence's and Barbario's were examined.

1. In comparison with each other.
2. As to their action on fresh and old stains.
3. On treated stains (washed).
4. On prostatic fluid obtained by prostatic massage.
5. On spermatozoa obtained direct from the seminal vesicles.

Summary of results in regard to macerating fluids.

As regards actual disolving power 1% Sodium Hydroxide is the most powerful, but it is so strong that it distorts the spermatozoa, even .1% is too strong.

Glycerine Pepsin is the best actual solvent but the preparation is hazy, and if stains are to be run in under the cover glass, consid--erable difficulty is experienced owing to the viscosity of the glycerine. If speed is not essential it has no points to recommend it in preference to

Roussin's Fluid. This fluid appears to be the best for general use, it gives a very clean preparation, and in the series of stains examined has shown spermatozoa as soon as, and usually before, any other fluid.

Glycerine and Water. Does not compare for a moment with either of the above and frequently fails to show spermatozoa.

Distilled Water. This is of very little use unless the spermatozoa are so easily seen that it does not matter what fluid is used. It evaporates quickly and its advantage lies in the fact that on reflooding the slide spermatozoa may be washed out into the open, and that then the spermatozoa are more easily seen.

Pacini's Fluid. This is no better than water.

Acetic acid (.3%). This appears to be quite good but it evaporates rapidly and requires constant renewal, so it cannot be left to macerate over night.

Erythrosin in Ammonia. This appears to have very little, if any, solvent action as it dries too quickly. The increased ease and rapidity with which spermatozoa are found is apparently due to its staining action, and to its effect as a background.

Sodium Chloride (.9%). This has no advantage over water, as is shown in the section on spermatozoa. When it dries it leave an untidy mass of crystals behind.

Hydrochloric acid (1in700=Ungar). This is practically the same as water.

The method of Ungar. Of touching a slide with the material after several hours soaking usually gives negative results, but when posit--ive the spermatozoa are very easily seen.

Action of solvents on spermatozoa.

Pure films of spermatozoa were made direct from the seminal vesicles and the effect of various solvents tried.

Water. Ungar states that the heads of spermatozoa are broken off by the mechanical action of the water which causes them to swell up, it is presumably on this account that Normal Saline is suggested as a solvent.

A smear from the seminal vesicles was examined under a cover glass, water was then run in and it was again examined, the heads were swollen up but no decrease in the number of intact spermatozoa was seen. The slide was allowed to dry and then reflooded, no difference could be made out and no debris or detached heads were seen; this process was repeated daily forfourteen days with the same result, a week was then allowed to elapse, the slide was reflooded again and a similar result obtained.

Saline. This has no advantage over water and on drying leaves a deposit of crystals.

Heat. Heating in water until steam rises does not apparently affect the spermatozoa.

Sodium Hydroxide (.5%). Spermatozoa were unrecognisable in two to three minutes.

Acetic acid (.3%). The spermatozoa are unaffected in the time the preparation takes to dry, thid is not very long.

Erythrosin in Ammonia. Spermatozoa were practically unaffected the stain dries very quickly, a few tails were seen to be twisted up. From their action on the stains Mitto Z, the three fluids Roussin's, Glycerine Pepsin, and Glycerine and Water appeared to be the most satisfactory, spermatozoa were mounted in these three fluids, placed under separate microscopes and left there, so the same area was examined each time. They were examined daily and the results are recorded below; The first to disappear were those mounted in Roussin's fluid, this was after fourteen days; after a week the only change was that they were fainter, and they could be recognised without difficulty at the end of ten days.

Spermatozoa mounted in Glycerine and Water.

Day 1st Spermatozoa well seen.

2. No change.

3. No distortion, well seen.

4. Not so well seen.

5. Fainter.

6. No change.

8. No change.

10. Still quite easily seen.

12. Ditto.

14. Getting rather faint.

16. Faint but perfectly clear and easily seen, not distorted or eroded.

Spermatozoa mounted in Roussin's fluid.

Day 1st. Spermatozoa well seen.

2. Rather fainter, no distortion.

3. Much fainter.

5. No change.

7. No change.

10. Very faint but still recognisable.

12. Ditto.

13. Some have disappeared, others faint outline only can be seen whilst some are quite distinct though faint.

14. Spermatozoa faintly made out, doubtful if they could be recognised in a teased preparation of cloth.

15. One spermatozoon can be faintly made out on carefully focussing.

Spermatozoa mounted in Glycerine Pepsin.

Day 1st. Well seen but do not stand out so well as in the other two preparations.

2. Same as Roussin's.

3. Fainter but shape perfect.

4. Much fainter.

8. Still easily seen but fainter.

10. Getting gradually fainter.

13. Some tails are hard to see.

14. Still quite well seen.

STAINING.

The effect of various stains was tested on an aqueous solution of spermatozoa obtained from the seminal vesicles. The results are given below.

Leishman. Not good, and in view of its expense and the much better results with other stains, it can be discarded.

Ziehl Neilsen The spermatozoa are not acid fast and the plain C carbol fuchsin, as recommended by Dr Goldsmid of N.S.W is to be prefered.

Gram. stains quite well but other methods are better.

Picro-Carmine. Very good but cannot be used if the stain is mounted in Roussin's as a thick deposit forms.

Gentian Violet. Stains very well indeed.

Iodine in Pot: iodidi. This appears to be one of the best stains to use, the spermatozoa are stained a deep brown and the tails are very easily seen.

Erythrosine in Ammonia. A very good stain.

Eosin and Methyl Green. Fairly good, but as a method of making the spermatozoa catch the eyeit is poor in comparison w with most of the above. The advantage claimed for it of differential staining seems to be out of place, if the spermatozoa are so distorted that they cannot be recognised by their very distinctive shape, it is safer not to regard them as such. The stain is not so good s as some others.

Methyl Green. Fairly good.

Carbol Fuchsin. Very good indeed.

Dried Watery Preparation. This makes the spermatozoa much more easily seen but unless the spermatozoa are very plentiful, water as a macerating fluid is not to be recommended.

The best stains are Iodine in Pot:Iodide, Erythrosine in Ammonia, Gentian Violet and Carbol Fuchsin.

These stains have been selected as those giving the best results with water, it is as well to see if the macerating fluids which are likely to be used have any effect on them. It has been stated above that Roussin's fluid is the best for general work.

The following is the result of adding Roussin's fluid to the stained spermatozoa or of adding the stain to Roussin's fluid.

Iodine in Pot:Iodide. Nil, but forms crystals with semen.

Erythrosin in Ammonia. A few crystals may form but usually there is no apparent change. If the Erythrosin be left on the film however, the Ammonia evaporates and a thick deposit forms.

Gentian Violet. Staining rendered rather fainter.

Carbol Fuchsin. Preparation decolourised.

Picro-Carmine. An immediate thick precipitate.

Erythrosine (aq: sol:) A thick precipitate.

Eosin and Methyl Green. A slight black deposit.

Of the four stains previously mentioned, one of them, Carbol Fuchsin gives a precipitate with Roussin's fluid, as it possesses no advant--ages over the others it may be dismissed. Of the three left, Gentian Violet makes a messy slide, black deposit adhering to any threads. Erythrosine in Ammonia appears to be the most siutable but the choice between it and Iodine will depend on the individual worker.

CHEMICAL TESTS FOR SEMEN.

Florence's and Barbario's reactions were tested with various stains,

1. Fresh stains.
2. Stains washed in hot water.
3. Stains washed with hot water and soap.

In addition the reaction was noted with

1. Spermatozoa direct from the seminal vesicles.
2. Prostatic fluid from three different cases, obtained by prostatic massage. Before the test was performed the slides were examined for spermatozoa, only a few (2to5) were found in each film.

The following is a tabulated list of the results obtained:-

Material tested.	Florence.	Barvario.
spermatozoa in water.	+	+
Prostatic fluid from massage.	+	+
Stain "Z" (3 months old).	+	−
Stain "Q" (22 years old).	+	−
Stain "T" (fresh).	+	+
Stain "Y" (washed in hot water).	+	+
Stain "X" (:: :: :: ::)	+	−
Stain "V" (:: :: :: ::)	−	−
Stain "S" (:: :: :: ::)	+	−
Stain "R" (washed in soap and water)	+	−

Barbarios reaction is much more easily lost than that of Florence, this conforms Welsch's statement(Archives Internationales de Medecine Legale, 1912.) that after five minutes washing Barbario's reaction was negative,whilst Florence's reaction remained for twenty minutes. Positive results were obtained both with preparations from the vesicles and with prostatic smears, the number of spermatozoa in the latter were so few that they could not have produced the very strong positive result obtained.

WASHING.

Dr Maidlow of Ilminster related a case in 1903 where he found sper--matozoa on a sheet after it had been washed. Dr Smith in Taylor's Medical Jurisprudence states that this only proves careless or incomplete washing.

Welsch[7] states that a few may be found after twenty minutes boiling but that a careful washingin soapy water renders them unrecognisable.

A fresh seminal stain was taken, part removed and thoroughly washed in hot water for five minutes, another part was washed with soap and hot water for five minutes; the stained linen, roughly four inches square, was rubbed together, rinsed, and in every way thoroughly washed, when dry no trace of the stain could be seen either by transmitted or reflected light.

Stain "T"(a). After washing in hot water for five minutes, spermatozoa were found after a long search, complete and quite definite.

Stains "V", "W", "X", "Y", were also washed in hot water, spermatozoa were found in each case but only after a long search, and in one case could not be found in the unstained preparation.

Stain "T"(b). Washed with soap and hot water for five minutes, The preparation was mounted in Roussin's fluid, no spermatozoa were seen in unstained slides, when stained two spermatozoa were seen after a search extending over days.

From these results it wauld seem that it is always advisable to examine stains even if they have been subjected to a washing with soap and water, provided of course that the position of the stain is known.

AGE. It is interesting to note that undisputable spermatozoa were found in one of the stains that was twentytwo years old.

CONCLUSIONS.

From amongst the various fluids advised for macerating purposes, two stand out preeminent, they are Roussin's fluid and Glycerine Pepsin.

If the stain is fresh and the spermatozoa are numerous it makes very little difference what fluid is used, as the stain becomes older or not so rich in spermatozoa, these two fluids begin to stand out in comparison to the others, until, at the other end of the scale, where the only spermatozoa remaining are those which are firmly fixed and therefore require more powerful solvents to detach them, these two are the only solvents that yield spermatozoa.

The next point to consider is, what is the best technique to employ?

Everyone seems agreed, that the fabric to be examined should be allowed to soak for some time in the macerating fluid, before anything further is done; the time varies from fifteen minutes advocated by Guy and Ferrier, to five hours recommended by Ungar. It would seem that the period depends on what is to be done with the material afterwards. If, as in the method used by Ungar and Glaister, several slides are to be touched with the material and then examined, then the longer the fabric soaks the better; if on the other hand the material is to be teased out with fine needles and mounted in the macerating fluid, prolonged soaking is unnecessary as the process is continued after mounting. The actual time will

depend on the fluid used and the material which is stained; with Roussin's fluid or with Glycerine Pepsin the material will be found to disintegrate, with very gentle teasing, in the course of a few minutes. There need be no hesitation about leaving the prepar--ations mounted in either Roussin's fluid or Glycerine Pepsin as even unprotected spermatozoa remain unaffected for a week or ten days in these fluids. If the spermatozoa are not found fairly readily it is advisable to leave the slide to macerate and examine again the next day.

Concerning the course to be followed after maceration there are no two ways. If the spermatozoa are plentiful they will be found whatever course is followed, if they are few, then hundreds of slides may be touched without leaving spermatozoa on them, but if there are spermatozoa present, then by teasing and mounting the fabric, whether spermatozoa are seen or not, the observer is at least searching a slide that contains them. As a striking method of demo--nstrating spermatozoa the first method is excellent, and the spermat--ozoa stand out well on a clear field, but it is a "hit or miss" method and one not to be recommended for routine use.

As regards staining, several methods are excellent when used with spermatozoa alone, but when used with a teased preparation to which deposit may cling, or when used with acids which may decolourise them, the number is considerably reduced.

The two best stains are Erythrocine in Ammonia and Iodine in potassium Iodide. Both these stains are good and very useful. It seems best to examine the unstained slide first, then, if necessary, stain by running in a small ammount under the cover glass and drawing it through with blotting paper. Their use is well demonstrated in stains"X" and"Y" were spermatozoa could not be found until the slides were stained. WASHING. Washing removes most of the spermatozoa but not all. Natur--ally prolonged washing should eventually get rid of everything, but it has been shown (stains"R" and"S") that even after vigorous rubbing and washing in hot water and hot soap and water, spermatozoa can be detected intact and recognised with certainty. There is no doubt that if the position of the stain be known, positive results may be obtained even after a garment has been washed.

The chemical tests of Florence and Barbario are an encouragement to further effort, if positive, when spermatozoa are hard to find; Florence's test is the more constant, Barbario's is not so certain and is easily destroyed by washing.

The reaction seems to be given by both the prostatic and vesicle components of the semen.

SUMMARY.

1. The following method should be used as a routine method.
   (a) Soak the fabric in Roussin's fluid for a few minutes until the strands separate easily.
   (b) Gently tease a portion on a glass slide with fine needles.
   (c) Cover with a cover glass and examine. If spermatozoa are not seen fairly soon, put aside and examine in twentyfour hours.
   (d) If desirable stain with Erythrosine in Ammonia or Iodine in Potassium Iodide.
2. The above method should be supplemented by a preparation in Glycerine Pepsin, in the event of there being difficulty in finding spermatozoa.
3. The examination should be made daily for five or six days before a negative result is decided upon.
4. Washing of the garnents is not a contra-indication to examination, if the position of the stain can be located.
5. Florence's and Barbario's reactions are given by both the prostatic fluid and the spermatozoa.

REFERENCES.

1. Farnum,C.G.; Biologic Test for Semen. J.A.M.A. Dec 28, 1901.

2. Uhlenhuth; Nachweis von Menschen und Thierblut.Deut.med.Woch.P499, 1901.

3. Hoktoen; Specific Precipitin test for Human Semen. J.A.M.A. March11 1922.

4. E.Ungar; Zeitschr.f.Gerichtl. Med.,1887, 1,316.

5. Sutherland,W.D.; Blood Stains. Their detection and determination of their source. 1907.

6. Cordonnier and Muller; Etude Sur La Resistance des Spermatozoides au Lavage.
Annales de Medecine Legale, Vol 2, 1922.

7. Welsch; Archives Internationales de Medecine. 1912.

8. Taylor; Principles and Practice of Medical Jurisprudence. Vol 1, seventh edition, 1920.

9. J.Dixon Mann; Forensic Medicine and Toxicology. 1914.

10. Guy and Ferrier; Principles of Forensic Medicine.1895.

11. Peterson and Haines; Text Book of Legal Medicine and Toxicology 1903

12. Draper; Text Book of Legal Medicine.1905.

13. Glaister; Forensic Medicine and Toxicology.1921.

A P P E N D I X .

Laboratory notes on macerating fluids.

Appendix.

STAIN "Z". Summary.

With the exception of one slide where spermatozoa were found straight away, evidently by chance, no definite spermatozoa were found free, although they could be made out in the clot after a long search in several cases. These preparations all contained a fair amount of clotted matter on which the dissolving power of the various fluids could be tested.

Sodium Hydroxide. As an actual solvent Sodium Hydroxide is the best, it disolves the clot in about fifteen minutes but it distorts the spermatozoa.

Glycerine Pepsin. This is the best of the other solvents, the clot begins to soften in three hours and is breaking up fast in twentyfour. The preparation is rather hazy. Definite spermatozoa were not seen.

Roussin's Fluid. This is much slower in its dissolving power twentyfour to fortyeight hours being necessary. It has the advantage that the preparation remains very clear, spermatozoa were definitely recognised and it appears to be more suitable than the previous one.

Glycerine and Water. This comes next in dissolving power.

Pacini's Fluid. This follows but both are very poor in comparison to the previous fluids.

Distilled Water and Erythrosine in Ammonia. Appear to have no effect on the clot whatever, the water dries and in the reflooding some spermatozoa may be washed out. The Erythrosine by staining makes the spermatozoa more easily recognised in the clot.

Appendix.

Ungar's Method . gave no results.

STAIN "Z".

A stain on a cloth coat from a case of indecent assault by a man aged seventy. The stain was three months old.

The stain was moistened with the maceratingfluids used and after a few minutes was teased with fine needles,mounted in the fluid,and examined after 3, 24, 48, and 72 hours. The spermatozoa were very scanty and special attention was paid to the breaking up of the clot. In the case of the Hydrochloric acid in water the technique of Ungar was followed,namely to allow the substance to soak for five hours and then gently touch a slide with it. The rest of stains were treated in a similar manner.

STAIN "Z".

Macerating fluid. PACINI'S FLUID.

Hours.

0. A lot of debris and clot, two free spermatozoa.

3. No change.

24. Clot not breaking up.

48. Clot broken into clumps, no spermatozoa.

72. Clot not beraking up further,two spermatozoa seen at the beginning

STAIN "Z".

Macerating fluid. 1% SODIUM HYDROXIDE.

clot disolves rapidly but spermatozoa are distorted.

Appendix.

STAIN "Z".

Macerating fluid. DISTILLED WATER.

Hours.

0. No spermatozoa found, a large amount of clot present.

3. No change.

24. Slide had dried, it was reflooded with water, one spermatozoon was washed out with some debris, no apparent effect on clot.

48. No spermatozoa seen, no change in the clot.

72. No spermatozoa identified, practically no change in the clot.

STAIN "Z".

Macerating fluid. GLYCERINE AND WATER.

Hours.

0. No spermatozoa seen, large amount of clot present.

3. No change.

24. Bodies seen which are probably spermatozoa.

48. No spermatozoa, clot disolving a little.

72. Clot breaks up readily on pressing.

96. No spermatozoa seen, part of clot broken up fairly well.

STAIN "Z".

Macerating fluid. ERYTHROSINE AND AMMONIA.

Hours.

0-48. No effect on clot, spermatozoa made out in the clot.

STAIN "Z".

Macerating fluid. HYDROCHLORIC ACID (1in700).

Ungar's Method. No spermatozoa found.

Appendix.

STAIN "Z".

Macerating fluid. ROUSSIN'S FLUID.

Hours.

0. One spermatozoon made out in the clot.

3. Clot becoming clearer, clot softening a little.

24. Not much difference.

48. Clot dissolving fairly well, spermatozoa recognised in the clot.

72. No change.

96. Clot very slowly dissolving.

120. Clot slowly breaking up, no free spermatozoa seen.

STAIN "Z".

Macerating fluid. GLYCERINE PEPSIN.

Hours.

0. No spermatozoa seen, fair amount of clot present.

3. Clot breaking up.

24. Clot hazy, breaking up fast, no spermatozoa.

48. Condition slowly progressing, one or two doubtful spermatozoa seen

72. The greater part of the clot has gone leaving a mass of debris, No spermatozoa found.

STAIN "Z".

Macerating fluid. 25% SODIUM HYDROXIDE.

The clot dissolves rapidly in fifteen minutes, free spermatozoa were seen but were too fuzzy to be definitely recognised.

STAIN "Y". Summary.

This stain had been washed and soaked in hot waterand its age was between twelve and fifteen years.

It was treated in the same way as stain "Z".On the second day each preparation was stained with Erythrosine and the results compared.

Roussin's. For the recognition of spermatozoa this appears to be superior to the others,it gives a very clearpreparation.

Glycerine Pepsin. It is a good solvent but the slide is hazy and the spermatozoa are not so easily seen.

Pacini's fluid. Appears to be no better than water.

Water,and Glycerine and Water.are far behind the first two mentioned.

Staining. The staining rendered the spermatozoa much more easily seen, and in the case of the last two solvents spermatozoa were not seen until the preparations had been stained.

Erythrosine in Ammonia.As a solvent it is very little use,possibly the slide dries too quickly. It stains the spermatozoa and also acts as a background,and on this account the spermatozoa are more easily seen. It is best to use it after the fabric has been macerated in some other fluid.

Ungar's Method. gave negative results.

Appendix.

STAIN "Y".

Macerating fluid. DISTILLED WATER.

Hours.
0. Nil.

3. Nil.

24. Nil.

Stained with Erythrosine. Spermatozoa recognised with difficulty after a long search.

STAIN "Y".

Macerating fluid. GLYCERINE AND WATER.

Hours.
0. Bodies (probably spermatozoa) seen lying along the fibres.

3. No free spermatozoa.

24. Ditto.

Stained with Erythrosine. Spermatozoa found almost at once.

STAIN "Y".

Macerating fluid. ROUSSIN'S FLUID.

Hours.
0. Several spermatozoa seen lying along the sides of the fibres.

3. One free spermatozoon seen.

24. Spermatozoa easily made out, preparation very clear.

Stained with Erythrosine. Spermatozoa seen immediately.

Appendix.

STAIN "Y".

Macerating fluid. GLYCERINE PEPSIN.

Hours.
0. Nil.

3. Nil.

24. Spermatozoa seen with difficulty.

Stained with Erythrosine. Spermatozoa seen fairly easily but not so clearly as with Roussin's fluid.

STAIN "Y".

Macerating fluid. PACINI'S FLUID.

Hours.
0. Two spermatozoa seen lying on fibres.

3. Ditto.

24. There is a deposit of crystals.

Stained with Erythrosine. No spermatozoa seen, there is a deposit on the slide.

STAIN "Y".

Macerating fluid. ERYTHROSINE IN AMMONIA.

Hours.
0. Free spermatozoa seen.

3. Ditto.

24. Free spermatozoa seen but not so easily as when the preparation is macerated with another fluid before staining. Its solvent action appears to be very poor.

Appendix.

STAIN "Y".

Macerating fluid. HYDROCHLORIC ACID (1in 700).

Slide touched with material after macerating for five hours, examined, nothing found.

Appendix.

STAIN "X". Summary.

This stain was of a similar nature to stain "Y". It had been washed and soaked in hot water, and was from twelve to fifteen years old.

In unstained preparations no spermatozoa were found. On staining spermatozoa were found with Roussin's fluid and with Glycerine Pepsin, with the latter they were rather more easily found, but the preparation was stained twentyfour hours later

STAIN "X".

Macerating fluid. GLYCERINE PEPSIN.

Hours.
0-48. Nothing.

72. Clumps of fibres breaking up.

Stained with Erythrosine. Free spermatozoa found in the debris, seen rather more easily than with Roussin's, but examined 24 hrs later.

STAIN "X".

Macerating fluid. ERYTHROSINE IN AMMONIA.

Hours.
0. Spermatozoa seen on fibres.

3-72. No change.

STAIN "X".

Macerating fluid. HYDROCHLORIC ACID (1in700).

Ungar's Method. No spermatozoa found.

Appendix.

STAIN "X".

Macerating fluid. GLYCREINE AND WATER.

Hours.

0. One or two probable spermatozoa made out adhering to the edge of the fibres.

3. No change.

48. No change.

Stained with Erythrosine. No free spermatozoa could be found but some could be made out with difficulty, attached to the fibres.

STAIN "X".

Macerating fluid. ROUSSIN'S FLUID.

Hours.

0. One or two doubtful spermatozoa seen on the fibres.

24. No change.

48. No change.

Stained with Erythrosine. Spermatozoa recognised after a long search.

STAIN "X".

Macerating fluid. DISTILLED WATER.

Hours.

0-48. Nothing was found after fortyeight hours.

Appendix.

STAIN "W". Summary.

This stain had been washed and soaked in hot water, its age was between twelve and fifteen years .

There appeared to be practically no difference in this case between Roussin's fluid and Glycerine Pepsin, the other methods tried ended in failure.

STAIN "W".

Macerating fluid. PACINI'S FLUID.

Hours.
0.-48. Nothing was seen after fortyeight hours.

STAIN "W".

Macerating fluid. DISTILLED WATER.

Hours.
0-48. Nothing was seen after fortyeight hours.

STAIN "W".

Macerating fluid. HYDROCHLORIC ACID (1in700).

Ungar's Method. No spermatozoa were seen.

Appendix.

STAIN "W".

Macerating fluid. GLYCERINE AND WATER.

Hours.
0-48. Nothing seen after fortyeight hours.

STAIN "W".

Macerating fluid. ROUSSIN'S FLUID.

Hours.
0. Nothing.

3. Nothing.

24. Two spermatozoa seen lying along fibres.

48. Free spermatozoa seen but only after a very long search.

Stained with Erythrosine. Ditto .

STAIN "W".

Macerating fluid. GLYCERINE PEPSIN.

Hours.
0. Nil.

3. Nil.

24. Spermatozoa seen lying along fibres.

48. Free spermatozoa seen.

Stained with Erythrosine. Ditto.

Appendix.

STAIN "V".

This stain was washed and soaked in hot water, it was between twelve and fifteen years old.

The Glycerine Pepsin preparation seemed to show more free spermatozoa than that of Roussin, but they were harder to recognise; the preparation was not so clear and not so easily stained.

Ungars Method in this case showed three spermatozoa.

STAIN "V".

Macerating fluid. GLYCERINE PEPSIN.

Hours.

0. Nil.

3. What appear to be free spermatozoa seen but not definite.

24. Nothing.

48. Free spermatozoa seen, not clearly, preparation is hazy and spermatozoa are not easily recognised.

Stained with Erythrosine. The spermatozoa are not so easily recogn--ised as with Roussin's.

STAIN "V".

Macerating fluid. HYDROCHLORIC ACID (1in700).

Ungar's Method. Three spermatozoa were seen.

Appendix.

STAIN "V".

Macerating fluid. ROUSSIN'S FLUID.

Hours.
0. Nil.

3. One or two almost free spermatozoa seen.

24. One free sprematozoon seen, one or two adhering to the fibres.

48. Ditto.

Stained with Erythrosine. A very few free spermatozoa seen.

Appendix.

STAIN "T".Summayy.

This was a recent stain. Spermatozoa were found whatever method was employed,they were most easily seen by Roussin's and Ungar's methods. Glycerine Pepsin was very good but not so clear as Roussin's. With Water, and Glycerine and Water, spermatozoa were only found with difficulty.

STAIN "T".

Macerating fluid. DISTILLED WATER.

Hours.
0. Spermatozoa were not well seen,two were seen free but only after a long search.

Stained with Florence's reagent. Easier to look for but the result the same.

STAIN "T".

Macerating fluid. GLYCERINE AND WATER.

Hours.
0. Result very much the same as with Water.

STAIN "T".

Macerating fluid. HYDROCHLORIC ACID (1in700).

Ungar's Method. Many spermatozoa easily seen,nothing could be better than this result.

Appendix.

STAIN "T".

Macerating fluid. ROUSSIN'S FLUID.

Hours.

0. A large number of spermatozoa were seen immediately, clearly, and easily, there was no difficulty at all.

Stained with Florence's reagent the spermatozoa stood out well but were not recognised any easier.

Four different preparations of this stain were mounted in Roussin's fluid and in each case spermatozoa were seen almost as soon as the slide was examined.

STAIN "T".

Macerating fluid. GLYCERINE PEPSIN.

Hours.

0. Spermatozoa were seen fairly quickly, but not so quickly or so well as with Roussin's which is much cleaner and clearer, the glycerine preparation being rather hazy; in addition the Roussin's preparation is easier to work with when running stains under the cover glass.

Appendix.

STAIN "Q". Summary.

A stain on red flannel from a case of criminal assault dated 5:12:99. Twenty two years old.

Roussin's fluid is without doubt the most satisfactory in this case. Pacini's fluid appears to be no better than water.

It is interesting to note that the stain was twentytwo years old.

STAIN "Q".

Macerating fluid. PACINI'S FLUID.

Hours.

0. Nil.

3. One or two doubtful spermatozoa seen adhering to the fibres.

24. Nil.

STAIN "Q".

Macerating fluid. DISTILLED WATER.

Hours.

0. Nil.

3. Doubtful spermatozoa seen on fibres and on clot.

24. One spermatozoon seen.

Appendix.

STAIN "Q".

Macerating fluid. GLYCERINE AND WATER.

Hours.
0. Nil.

3. A few doubtful spermatozoa seen on fibres.

24. One definite spermatozoon seen,

STAIN "Q".

Macerating fluid. ROUSSIN'S FLUID.

Hours.
0. Not examined.

3. Numerous free spermatozoa seem after a short search, a very clear, clean preparation.

24. Spermatozoa easily seen.

STAIN "Q".

Macerating fluid. GLYCERINE PEPSIN.

Hours.
0. Nil.

3. One almost free spermatozoon, two unmistakable ones seen on fibres.

24. One free spermatozooz seen .

Appendix.

STAIN "N". Summary.

Roussin's fluid appears to be the most satisfactory, Glycerine Pepsin is not so clear, but except for this, is almost as good. Water, and Glycerine and Water, were not much use.

Glaister's Method was tried; out of the six slides touched there was one positive result.

STAIN "N".

Macerating fluid. DISTILLED WATER.

Hours.
0-24. Nil.

48. Two probable spermatozoa seen on fibres, a very poor result.

STAIN "N".

Macerating fluid. GLYCERINE AND WATER.

Hours.
3. One spermatozoom seen in the clot.

24. No change.

48. No definite spermatozoa seen, several doubtful ones found.

STAIN "N".

Macerating fluid. ACETIC ACID (.3%).

Glaister's Method. Six slides were made, one spermatozoon was found, one doubtful one seen, the other four slides were negative.

Appendix.

STAIN "N".

Macerating fluid. ROUSSIN'S FLUID.

Hours.
0. Not examined.

3. Spermatozoa found after a long search.

24. Ditto.

48. Spermatozoa found more easily.

STAIN "N".

Macerating fluid. GLYCERINE PEPSIN.

Hours.
0. Nil.

3. Spermatozoa found after a long search.

24. Ditto.

48. Spermatozoa seen but preparation is not so clear as Roussin's.

STAIN "N".

Macerating fluid. ACETIC ACID (.3%).

Hours.
0. Nil.

3. Many bodies seen, probably spermatozoa.

24. Ditto.

Appendix.

STAIN "M". Summary.

Stain from a pair of linen draws eleven years old, 26:2:11.

This bears out the other stains in that Roussin's fluid and Glycerine Pepsin are the best solvents. Acetic acid appears quite good but it dries up easily and has to be renewed constantly.

STAIN "M".

Macerating fluid. DISTILLED WATER.

Hours.
24. One very doubtful spermatozoon seen.

Macerating fluid. GLYCERINE AND WATER.

3. Several very doubtful spermatozoa seen.

24. Ditto.

Macerating fluid. ROUSSIN'S FLUID.

3. Spermatozoa seen, not easily.

24. Definite spermatozoa found with difficulty.

Macerating fluid. GLYCERINE PEPSIN.

3. One spermatozoon seen.

24. Several spermatozoa seen after a long search.

Macerating fluid. ACETIC ACID (.3%).

24. Several spermatozoa seen.

Macerating fluid. ACETIC ACID (.3%).

Glaister's Method. One doubtful spermatozoon in the six slides.