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Blood and Semen Stains on Outer Clothing and Shoes Not Related to Crime: Report of a Survey Using Presumptive Tests

In many crimes blood and semen stains play an important part in the conclusions that a forensic scientist will present in court as evidence. There is, however, an absence of data relating to the frequency of occurrence of blood and semen stains on clothing unrelated to crime. This was discussed by Tryhorn [*1*], who said:

> One need only consider the frequency with which evidence regarding blood and semen stains is produced in court, to realise the need for data relating to the relative frequency of occurrence of such stains on garments in no wise related to crimes; for example, on one hundred garments chosen at random from miscellaneous sources, how many would show blood stains, how many semen stains? Questions such as these must arise in court, and answers based on experimental investigation would prove of considerable value in assessing evidence of this type.

It is interesting how similar the above observations are to the justification which was drawn up for this current project and perhaps a little depressing that Tryhorn's questions, which were formulated in 1935, have not yet been answered.

Since its inception in 1967, the Home Office Central Research Establishment has made available staff and facilities to answer these types of questions and one of the functions of the Information Division of this establishment is to gather basic background data that will be of value to the practicing forensic scientist.

A survey of glass and paint fragments on 100 men's suits has already been carried out [*2*] and data collected recently from 1010 exhibits handled by the criminalistics section of a United Kingdom Home Office Regional Forensic Science Laboratory showed that 24% of the exhibits handled were examined for blood and 21% for semen. It is therefore clear that some information is required concerning the incidence of blood and semen stains on clothing unrelated to crime.

The collection of clothing from all sections of the community poses many problems for such a survey; for example, obtaining a random sample of female undergarments for examination would be very difficult. It was decided that the survey should be restricted to the examination of relevant but easily accessible clothing. A dry cleaning establishment with a radius of collection of approximately 20 miles was found to be a readily available source of men's jackets and trousers. The most available source of shoes was a small shoe-repair business with a radius of collection of approximately 5 miles. Men's jackets and trousers were examined for blood, men's trousers for semen, and shoes of

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both sexes for blood only. The clothing items could be visually examined and any nondestructive test conducted but no cutting of items was permissible. The use of methods such as microscopy for sperm identification and species determination of the bloodstains was impracticable in the two establishments and therefore the presence of blood and semen had to be detected by visual examination and simple presumptive tests.

In a casework situation, presumptive tests would not be acceptable as proof of the presence of either blood or semen. However, in a survey the requirements are less stringent as long as it can be established that the number of interferences which would be expected due to nonspecific reactions are small compared with the unavoidable statistical sampling errors. Then, presumptive tests are entirely acceptable and little is to be gained by more elaborate procedures.

Procedures Used in the Survey

Blood Presumptive Test—Kastle Meyer Reagent

The presumptive test reagent for blood is prepared by adding phenolphthalein (4 g), potassium hydroxide (40 g), and zinc dust (20 g) to distilled water (200 ml). The mixture is boiled until the pink coloration has practically disappeared, cooled, and stored in a refrigerator. Before use, an aliquot of stock solution is diluted with an equal volume of ethyl alcohol.

Semen Presumptive Test—Acid Phosphatase Reagent

The presumptive test reagent for semen is prepared by adding glacial acetic acid (10 ml) to distilled water (2000 ml). Anhydrous sodium acetate (24 g), alpha naphthyl phosphoric acid (2 g), and Fast Black Salt K (2 g) are then dissolved to produce the reagent, which is stored in a refrigerator and filtered before use.

Examination of Clothing

Twice weekly, on differing days over a period of eleven weeks, the dry cleaning establishment was visited and men's trousers and jackets were taken at random from the collection arriving for dry cleaning. Each item was laid out on a table, which was brightly illuminated with white fluorescent tubes, and thoroughly examined visually for blood on the outside, inside, and in all the pockets. Any suspicious areas of staining were rubbed with Whatman No. 1 filter paper and the area of rubbing on the filter paper tested with one drop of Kastle Meyer reagent followed by 1 drop of hydrogen peroxide (5.6% weight/volume). Altogether 100 jackets and 100 trousers were examined for blood and 58 of these were matching pairs, that is, suits.

100 pairs of trousers were examined for semen and the area tested was restricted to the inside and outside front of the trousers from the waist to the lower thigh. The trousers were made damp with a fine spray of water and then a large damp sheet of absorbent blotting paper was pressed firmly against the test area for 2 min. Relevant points of reference, such as the crotch and belt, were marked before the unfolded damp sheet was returned to the laboratory, individually wrapped in polythene, and sprayed with acid phosphatase reagent in a fume cupboard.

Once weekly on varying days over a period of three months, the shoe repairer was visited and shoes awaiting repair were examined. The whole surface of each shoe was examined visually for blood and then rubbed with a filter paper which was tested with

the Kastle Meyer reagent. In all, 50 pairs of men's shoes and 50 pairs of women's shoes were examined.

Record of Results

The location of each bloodstain on the garment was sketched and the maximum dimension and approximate area of the largest stain on each garment was recorded. A sketch was made on the form shown in Fig. 1 for each pair of trousers which gave

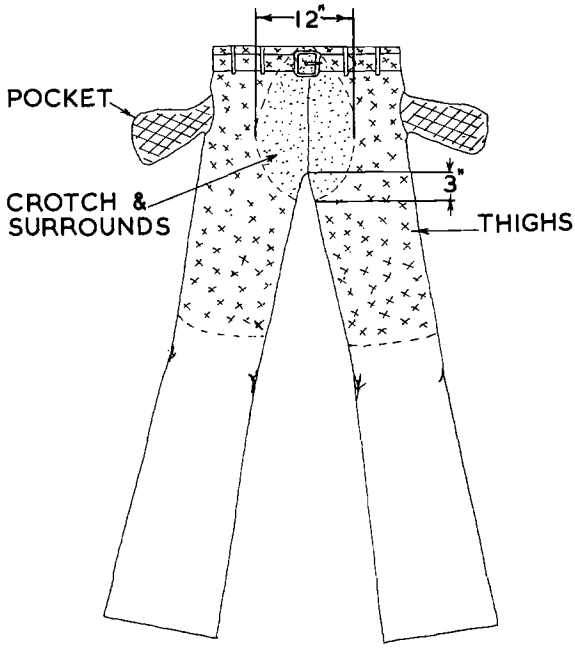


FIG. 1—*Definition of areas for the location of acid phosphatase activity on trousers.*

positive acid phosphatase reactions. The stains were also classified according to the intensity of color reaction with the acid phosphatase reagent as follows:

Intense—An area of color development which was visible within 5 s of spraying and developed into an area of intense color with well-defined outlines.

Moderate—An area of color development which was visible within 30 s of spraying and developed into an area of moderately intense color with fairly well-defined outlines.

Diffuse—An area of color development which was visible within 2 min of spraying and developed into an area of weak color with poorly defined outlines.

The maximum dimension and approximate area of the largest stain in the most intense color category was also recorded for each pair of trousers showing acid phosphatase activity.

Specificity of Presumptive Tests

Blood

The most widely used presumptive test for blood in the United Kingdom Home Office Regional Forensic Science Laboratories, as used in this survey, relies on the peroxidase activity of hemoglobin [*3*]. A comparison of available tests for blood has recently been reported by Owen [*4*] and possible interferences with the test have also been discussed [*3.5.6*]. Culliford and Nickolls [*7*] considered that in the hands of an experienced worker the two-stage test for peroxidase activity using benzidine can be regarded as specific for blood. Benzidine is now known to be carcinogenic and this precludes its use. The two-stage Kastle Meyer (phenolphthalein) test is an acceptable noncarcinogenic alternative. For the purposes of a survey of 200 items, a visual examination followed by a two-stage presumptive test for peroxidase activity was considered to be specific for blood in agreement with the conclusions of Culliford [*3*].

Semen

The most widely used presumptive test for semen, as employed in this survey, relies on the presence of the enzyme acid phosphatase [*8,9*]. The most suitable diazo salt for the test has been reported to be Fast Black[2] Salt K [*10*], although other salts have also been used [*11*]. Possible interferences with this test have been discussed by Kind [*12*] and also by Burgen [*13*].

Some investigators [*14,15*] state that a qualitative acid phosphatase test is insufficient to prove semen in the absence of other tests. Other workers [*16,17*] accept that in practice the acid phosphatase test is specific for seminal fluid.

Kaye [*18*] analyzed 36 different items ranging from vaginal fluid, urine, and blood to meat gravy, beet juice, and mayonnaise and found each to contain less than 5 King-Armstrong units of acid phosphatase per millilitre of concentrated pure substance, while the average seminal fluid sample contained 2500 units of the enzyme per millilitre. Fischer [*19*] reported that the acid phosphatase value of all body fluids was below 20 units per millilitre while that of the ejaculate varied from 400 to 8000 units per millilitre.

Schiff [*20*] concluded that while acid phosphatase was not unique to seminal fluid, the enormous amounts encountered compared to other body fluids and foods are unique and unequalled. Lundquist [*21*] came to a similar conclusion, namely that the acid phosphatase content of stains could be used as a specific test for the presence of seminal fluid.

The test for acid phosphatase used in this study is semiquantitative. In order to establish the suitability of the test for survey purposes, a comparative study was made of the acid phosphatase content of 50 samples of semen and 3 samples of urine, the latter being the body fluid thought most likely to appear on men's trousers.

The single-dimensional Laurell electrophoresis technique as described by Baxter [*22*] was used in the initial examination. The results obtained for the semen samples containing highest and lowest acid phosphatase contents are shown in Table 1, together with the results of two normal urine samples (Urines 1 and 2) and one urine sample (Urine 3) taken 5 min after ejaculation. The acid phosphatase content of these samples was measured using the sigma technique [*23*].

Stains were prepared on cotton cloth from the above samples. The semen samples were progressively diluted with physiological saline in order to produce stains at different

[2] Color Index Azoic Diazo No. 38.

TABLE 1—*Total acid phosphatase contents of two semen and three urine samples used to prepare stains.*

Sample	Total Acid Phosphatase, su/ml
Semen 1 (high)	42,600
Semen 2 (low)	9,600
Urine 1	0.7
Urine 2	9.5
Urine 3	13.6

but known concentrations of acid phosphatase. The urine samples were applied neat and progressively drop by drop on the same spot, allowing air drying between the application of each drop, so that the acid phosphatase concentration on the cloth progressively increased. The areas of the stains were calculated and the concentration of acid phosphatase present expressed in sigma units (su) per cm^2.

The stains were then treated by an identical procedure to that employed in the survey. The results for the seminal stains were used to convert the color categories intense, moderate, and diffuse semiquantitatively into units of acid phosphatase per cm^2, as shown in Fig. 2.

The urine stains, even after 50 drops had been applied on the same spot with air drying between each application, gave a maximum acid phosphatase concentration of 4.8 su/cm^2 and produced no visible color reaction within 2 min. Therefore, such urine stains would not have been recorded in the survey, which is in accordance with the results from the diluted semen samples shown in Fig. 3.

Thus, it was concluded that for the purposes of the survey the intense and moderate color categories are specific for fluids which originate in whole or in part from the prostate and that the diffuse category is not entirely specific for such fluids. In the absence of an examination for sperm, it cannot be established with certainty that the stains of prostatic fluid formed part of an ejaculate but it seems likely that in most cases they did so. In any event, seminal stains would normally be mapped using the acid phosphatase test prior to confirmation of the presence of sperm by microscopy in localized areas.

Results

Blood

Jackets—A total of 13 bloodstains was found on 5 of the 100 jackets examined. The position, maximum dimension, and area of the largest stain on each jacket are shown in Table 2.

Trousers—A total of 62 bloodstains was found on 16 of the 100 pairs of trousers examined. The position of these stains is shown in Table 3. The distribution of bloodstains among the trousers examined is shown in Fig. 4. The maximum dimension and area of the largest bloodstain on each pair of trousers are shown in histogram form in Figs. 5 and 6, respectively. The results for only 15 pairs of trousers are shown in Figs. 5 and 6 because one pair of trousers had a large area of bloodstaining extending over the legs and thighs which also contained fat, bone and pieces of flesh. These stains were

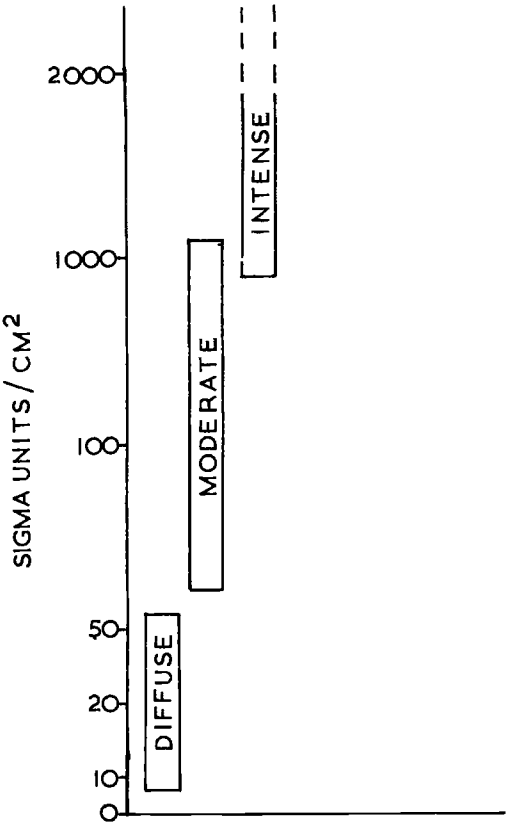


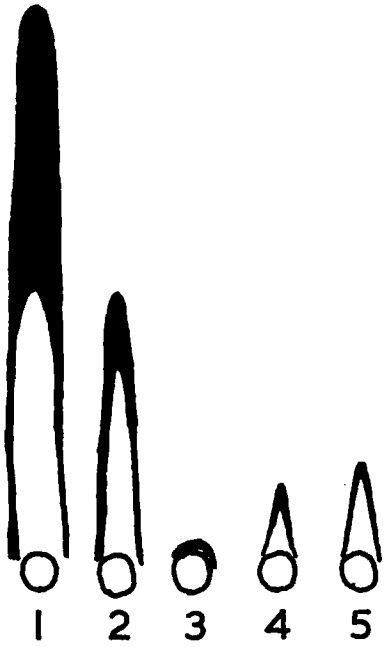
FIG. 2—*Semiquantitative relationship between intensity of color reaction and acid phosphatase concentration of prepared stains.*

unlikely to be of human origin and therefore this garment was excluded from the size distribution.

Shoes—One pair of men's shoes from the 50 examined gave a positive test for blood, although nothing was visible to the naked eye. On investigation this pair of shoes was found to be the property of the local butcher so the reaction was probably with blood of animal origin. One pair of men's shoes also gave a pink color before the hydrogen peroxide was added. No evidence of blood was found on the remaining men's shoes or on the 50 pairs of women's shoes.

TABLE 2—*Position, maximum dimension, and area of the largest bloodstain on five jackets.*

Area Observed	Bloodstains Found	Jackets in Which Bloodstains Were Found	Maximum Dimension, mm	Area, mm²
Lining, back	4	1	15	60
Lining, sleeves	8	3	4, 10, 31	8, 30, 403
Outside front, lapel	1	1	3	9
Others	nil	...	...	...



1 Semen 1 Dilution factor, 1:200
2 Semen 2 Dilution factor, 1:200
3 Urine 1 Dilution factor, nil
4 Urine 2 Dilution factor, nil
5 Urine 3 Dilution factor, nil

FIG. 3—*Single-dimensional Laurell electrophoresis of two semen and three urine samples.*

Semen

Areas of acid phosphatase activity were detected on 44 pairs of trousers using the test described. For 32 of the pairs of trousers examined the strongest reaction obtained was intense, for 5 pairs it was moderate, and for 7 pairs merely diffuse. These intense and moderate reactions, which are characteristic of prostatic fluid, were found on 37 of the 100 pairs of trousers examined. Eight pairs had stains on the outside only and 10 on the inside only. The remainder had staining which appeared on both sides.

TABLE 3—*Position of bloodstains on 16 pairs of trousers.*

Area Observed	Bloodstains Found	Trousers in Which Bloodstains Were Found
Outside front	17	5
Outside back	13	3
Inside front	6	5
Inside back	1	1
Pockets	25	7

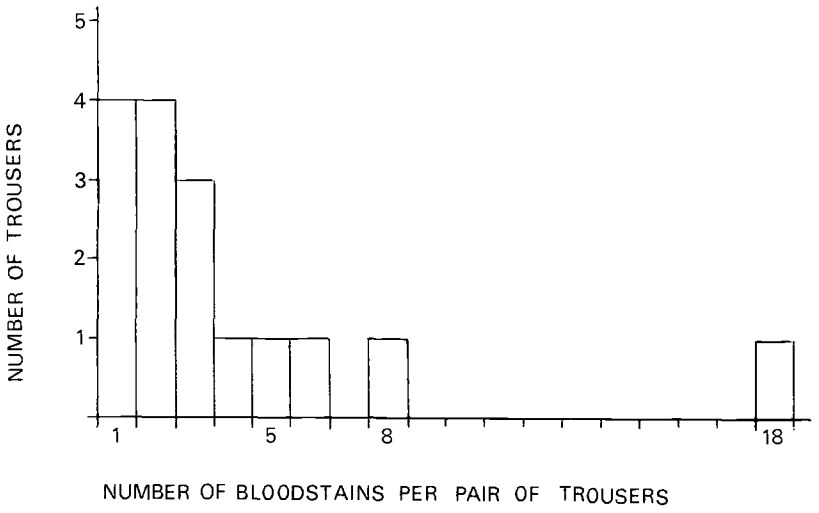


FIG. 4—*Distribution of bloodstains on trousers.*

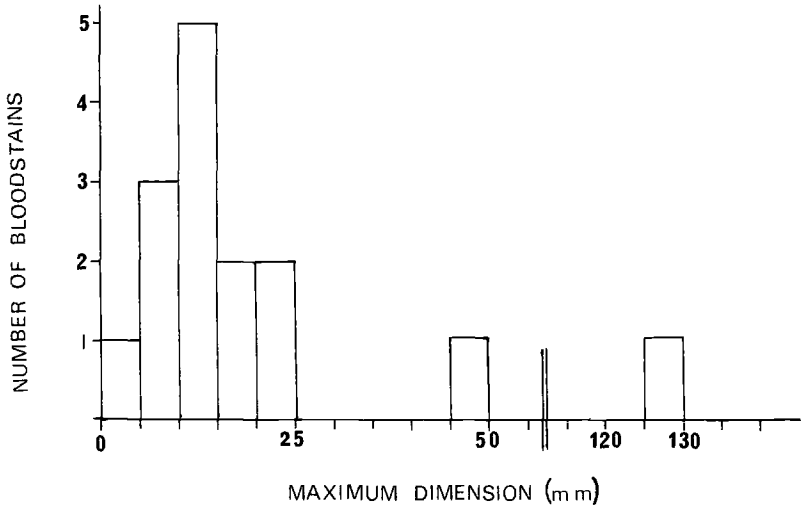


FIG. 5—*Maximum dimension of the largest bloodstain on each of 15 pairs of trousers.*

The position and intensity of color reaction for all the stains showing acid phosphatase activity are shown in Table 4. If a stain was found to extend over the crotch and thigh areas, a positive result was recorded in both positions. The maximum dimension and area for the largest stain in the most intense color category on each pair of trousers are shown in Figs. 7 and 8, respectively.

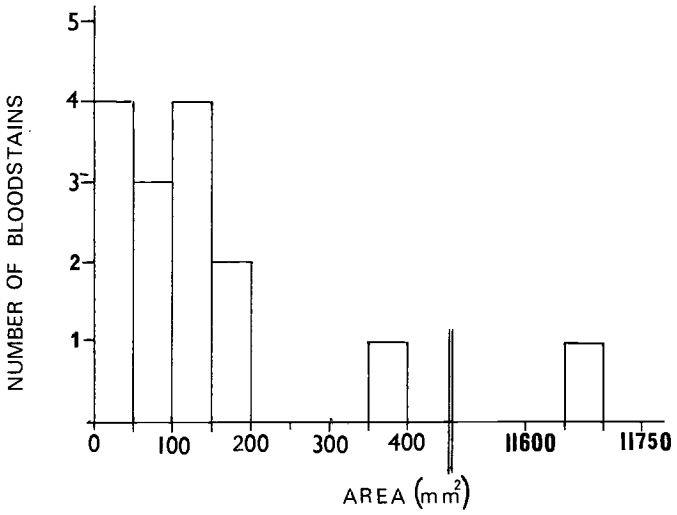


FIG. 6—*Area of the largest bloodstain on each of 15 pairs of trousers.*

Discussion

Blood

Although 13 bloodstains were found on jackets, 12 of these were on the jacket linings. The single remaining stain on a lapel was relatively small (9 mm^2) and represented the only bloodstain found on the outside of 100 jackets.

Of the 62 bloodstains found on trousers, 30 were on the outside, which is a much larger number than for jackets. If the null hypothesis is assumed, that jackets and trousers are in fact equally likely to be bloodstained, this can be tested using the Chi-square test. The value of Chi-square obtained is 4.8 with 1 degree of freedom, which is significant at the 5% level. Thus, there is significant evidence that a larger proportion of trousers are bloodstained than jackets.

TABLE 4—*Position and intensity of acid phosphatase color reactions observed on 44 pairs of trousers.*

	Stains			Trousers in Which Stains Were Found		
Area Observed	Intense	Moderate	Diffuse	Intense	Moderate	Diffuse
Outside front, thighs	20	20	17	15	9	16
Outside front, crotch and surrounding area	34	31	24	20	13	17
Inside front, thighs	19	11	16	17	9	16
Inside front, crotch and surrounding area	45	16	28	27	11	26
Pockets	0	3	0	0	2	0

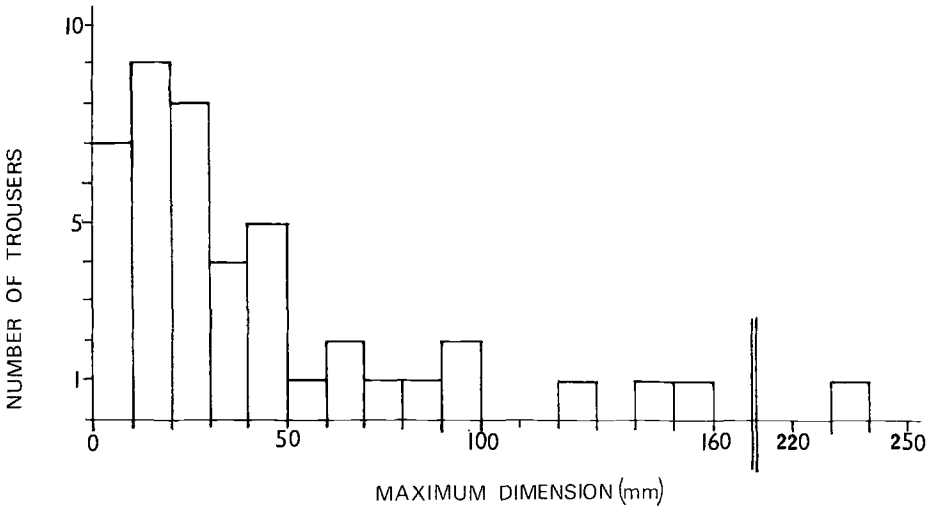


FIG. 7—*Maximum dimension of the largest stain in the most intense color category of acid phosphatase activity for 44 pairs of trousers.*

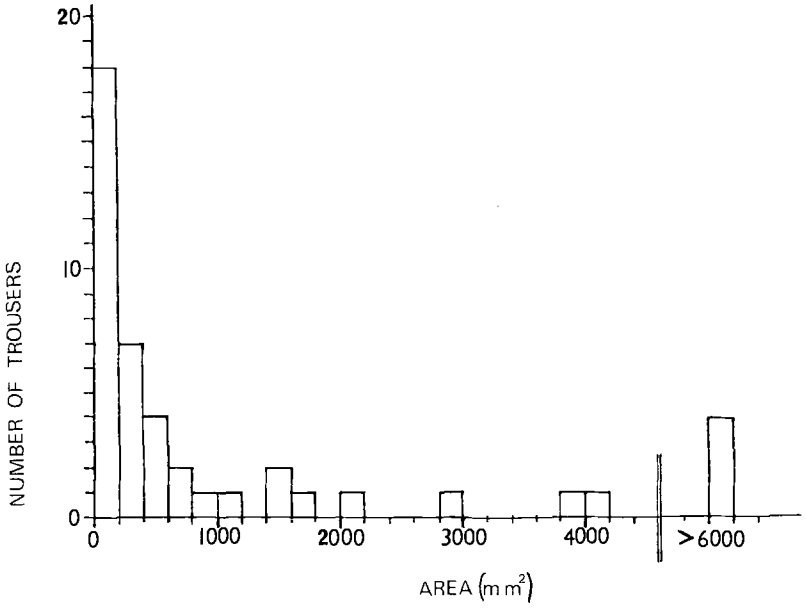


FIG. 8—*Area of the largest stain in the most intense color category of acid phosphatase activity for 44 pairs of trousers.*

One pair of trousers yielded 18 areas of bloodstaining. The largest stain had a maximum dimension of 130 mm and an area of 11,700 mm^2. This item exhibited heavy staining on the knees with corresponding "soak-through" on the inside. There were also bloodstains and smears on the front outside of both thighs, on the outside right buttock area and behind the left knee.

In this survey, although there is a tendency for blood-positive garments to show several bloodstains (Fig. 4), the largest stain was rarely more than 25 mm at its widest point or 200 mm^2 in area.

It could be suggested that this survey is biased because individuals are likely to send bloodstained garments for dry cleaning. In fact there were two extensively bloodstained pairs of trousers which may have been sent to the cleaners for this reason. The results for these garments would not influence the size distributions unduly because multiple stains on the same item were obviously associated and therefore only the largest stain on each garment was used. The clothing in the remainder of the survey showed only trivial areas of bloodstaining and was unlikely to have been taken to the cleaner for this reason.

This survey could be regarded as having set an upper limit for the incidence of bloodstains on the garments examined. The 95% confidence intervals for the incidence of bloodstains on the population of garments studied were calculated using the binomial distribution and were found to lie between 0 to 7% for men's shoes, 2 to 11% for jackets, and 9 to 25% for trousers.

Semen

The results show that 44% of the men's trousers examined had detectable acid phosphatase on them and that 32% showed at least one area of intense staining. Although the majority of these stains were less than 50 mm at the widest point and 600 mm^2 in area, some were much larger.

As would be expected, many stains were present in the area surrounding the crotch. However, a considerable number was either present on, or overlapped into, the thigh areas. Two pairs of trousers showed staining in the pockets.

In the survey, trousers which formed part of a suit were as likely to show the presence of acid phosphatase as those submitted for dry cleaning as single items.

As a result of the visual examination of the trousers, it was thought unlikely that items had been sent for cleaning because they were semen stained. The size distribution of the largest stain showing acid phosphatase activity for the twelve pairs of trousers in the moderate and diffuse color categories, was similar to the size distribution of the largest intense stain for all the other garments. The 95% confidence interval for the incidence of detectable acid phosphatase on the population of trousers examined, calculated using the binomial distribution, lies between 34 and 54%.

The size and number of stains showing acid phosphatase activity found in this survey suggests that the results for the routine examination of men's trousers for semen may need to be interpreted with great caution. Special circumstances pertaining to a particular case may nevertheless make this type of examination worthwhile.

Summary

One hundred men's jackets received at a dry cleaning establishment were examined for bloodstains and 100 pairs of trousers were also examined for blood and semen using simple presumptive tests.

Blood was found on 5 jackets and 16 pairs of trousers. Stains containing significant concentrations of acid phosphatase were detected on 44 pairs of trousers. Thirty-seven pairs showed sufficiently high concentrations of acid phosphatase to indicate that they contained prostatic fluid and therefore could well have been part of an ejaculate. The position, maximum dimension, and approximate area of the stains were recorded.

Although no visible bloodstains were detected on 50 pairs of men's shoes and 50 pairs of women's shoes arriving at a shoe repairer, one pair of men's shoes gave a presumptive test for blood.

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