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SOME PROBLEMS IN BLOOD TESTING AND GROUPING*

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A. Boyd Connors was a student at the School of Criminology when the research for this paper was being carried out.—EDRROR.

Several difficulties in routine application of blood testing methods (1), and particularly of blood grouping (2) have arisen in this and other laboratories. The reasons for some of these and methods of avoiding or correcting the difficulties need to be called to the attention of workers in crime laboratories. Recurrent questions that constantly arise in testimony, such as the effect of washing of cloth on the tests for blood are also of importance and should be answered to the extent possible. At least a portion of the answers to these problems are available and constitute the material of this contribution.

DETERGENT EFFECT ON BLOOD GROUPING

The entire practice of laundering has undergone a revolution in recent years with the increased use of automatic machinery and its demand for detergents which have now largely replaced soap as a laundry cleanser, and to a large extent as a household cleanser. The important point, as it affects the crime laboratory, is not the replacement of soap but the fact that soap required thorough rinsing to remove the excess before a satisfactory result was obtained. Many synthetic detergents do not require rinsing, or at least only mild rinsing, so that they are retained in garments, and in upholstery, rugs, and similar items as well. Not only the detergents, but such materials as Solium, a fluorescent blue-white dye, which actually dyes the white garment white, are forcing new concepts on the laboratory investigator. A strong white fluorescence no longer can be interpreted in terms of the material of which the cloth is made, or of significant foreign material on it.

Surface active agents such as the saponins have long been used for lysis of red blood cells in biological research. The action of detergents is very similar to that of saponins, and lysis of cells might be expected in the presence of excess detergent. This phenomenon was encountered in grouping blood by the inhibition method for blood stains soaked from a rug. It was not uniform, in that some spots did not produce lysis of the cells, but there was no clumping when clumping might have been expected, thus leading to a possible incorrect group. Tests for detergent were made in unstained areas of the rug, and it was possible to show that there was a very considerable

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amount of detergent present. Further, the diluted soakings from such areas also gave inhibition of cell clumping characteristic of the agglutinogens of human blood, even though no blood was present. Concentrated material led to lysis of the cells. As a check on this effect, a rug carrying no detergent was treated with a selected list of commercial detergents in selected areas. The results of testing these areas bore out the idea that the detergent was the cause both of the inhibition (dilute) and the lysis (concentrated). The material Miracle Foam, widely used on rugs and upholstery appeared to be most effective in producing these interferences.

Since this set of observations was made, similar difficulties have been reported from other laboratories, thus indicating that this type of difficulty may become frequent and widely troublesome. Preliminary work has been done in the effort to fractionate the agglutinogens from the detergent by alcohol precipitation methods, but limited success only has attended the effort. Further work along this line is contemplated because of the serious nature of the difficulty and its apparent increasing incidence. It should be emphasized that this effect makes even more critical the necessity for careful controls on unstained portions of the object tested.

STABILITY OF SODIUM PERBORATE

Sodium perborate was recommended (1) as a substitute for hydrogen peroxide, not only in the benzidine test, where it has often been employed, but also for other common color tests for blood, and especially for the luminol (3-aminophthalhydrazide) test by spraying of the reagent. Considerable variation in the intensity of this test has often been observed, though the maximum sensitivity that can be achieved is enormous. At times, the test as recommended failed almost entirely even with adequate quantities of known blood, leading to false negative reactions. This difficulty also, has been called to the authors' attention by other workers. At least part of the trouble was located readily when it was found that sodium perborate is not as stable as originally assumed. The first reagent that was encapsulated in a blood test kit continued to perform satisfactorily for about two years after which it no longer was effective when mixed with luminol and sodium carbonate. New lots of perborate were sometimes effective, and sometimes they also did not give a strong reaction. It was noted that in every instance of negative tests, the perborate showed a damp, caked appearance, and occasionally this was present in a new, unopened bottle of reagent. Relief to the difficulty was found by storing the dry reagent in a desiccator over dry calcium chloride when not in actual use. Under these circumstances, no false negative reactions have been obtained, though the sensitivity of the reagent has not always been as great as it is found when the reagent is optimal. No actual assay of oxidizing capacity of the perborate has been made, nor is it certain that other problems do not exist, but at least the most serious of them is capable of ready control. As has always been true with the luminol test, it is essential that the test be applied in as near absolute darkness as possible, and that the reagent be absolutely fresh.

DETECTION OF BLOOD AFTER LAUNDERING

The question has frequently arisen as to how much washing is required to prevent successful testing of blood on a cloth object. On the face of it, washing can certainly

remove blood sufficiently well to prevent any known test from being effective, since remaining blood is almost invariably soaked off with water or saline solution before carrying out a test. At the same time, it is common experience in the crime laboratory to have washed clothes yield definite blood tests from which has come the idea that blood can always be detected even after washing. In many instances, the blood is originally only present in spots or smears, and it is logical that the wearer might merely sponge the clothing with a wet cloth or similar object rather than to submit the entire garment to laundering. This would have the effect of spreading and diluting the blood rather than removing it totally, so it remains of even greater interest to make actual tests of the efficiency of laundering by various means.

To test this matter, seven cloths were taken for comparative testing as follows: 1. Cotton print; 2. Soft, medium weight cotton; 3. Wool blanket; 4. Rayon underwear; 5. Corduroy; 6. Light weight satin; 7. Chiffon. Each was stained with blood and subjected to various laundering operations in triplicate as follows:

a. Washing by hand, two minutes with White King soap solution, rinsing for one minute, no ironing.

b. Same as a. but with ironing after each washing.

c. Washing in Spindryer seven minutes with Surf detergent, rinsing for two minutes.

d. Same as c. but with ironing after each washing.

e. Commercial laundering with strong detergent, washing time seven minutes, rinsing time two minutes.

f. Same as e. but with ironing after each washing.

g. Commercial dry cleaning, washing and rinsing time, forty minutes.

h. Same as g. but with ironing after each cleaning.

Not only was each cloth subjected to triplicate tests, but every one was repeated by the method chosen until all blood tests were negative. The method of testing was to soak off the blood by the method described earlier with filter paper capillary action (3) followed by the phenolphthalin test which was proved most specific and sensitive. The luminol test was used to locate spots when necessary.

Without detailing the individual results, the comparative findings can be summarized as follows:

a. Cloths 1 and 2 held the blood most tenaciously, but in no case was a positive test obtained after the fifth consecutive washing. Cloths 6 and 7 were positive after one washing about half the time, but never after the second consecutive washing. Cloths 3, 4, and 5 were intermediate.

b. Dry cleaning was uniformly more effective in preventing positive blood tests than any type of washing, five out of seven cloths ordinarily giving positive tests after one cleaning, and not more than two or three after the second cleaning. None survived a third cleaning.

c. Ironing reduced the incidence of positive tests slightly, probably by fixing the stain by heat.

d. Only small and irregular differences were found between the different methods of water cleansing. Commercial laundering did not remove blood significantly better than the Spindryer, and only very slightly better than washing by hand for a shorter period.

The interpretation of the results summarized above in light of the method of removal and testing of the blood that followed each is not simple. The most conspicuous fact that emerges is that the type of cloth is the most important single factor, producing far greater differences than any other variable. Tightly woven cotton cloths held blood most tenaciously, with thin smooth cloths giving it up rather readily. It must also be concluded that the time of exposure of the blood to solvent is highly significant because the method of testing involved soaking of the blood with water traveling on filter paper. The method used (3) exposed the stained surface to a constantly renewed supply of fresh water over a considerable period of time, much longer than any of the washing operations. Otherwise, it was in all respects milder than the laundering. It was effective in recovering some blood that survived the more drastic but shorter cleaning. This points up the slowness of the solution process and gives significance to the old practice of soaking blood spots in cold water as a means of removing them.

It is probable that the effectiveness of dry cleaning in preventing blood tests as compared with wet washing was due to the denaturation of blood proteins to render them insoluble. This effect of organic solvents on protein solutions is well known. Heat also, as applied in ironing probably exerted the same general effect. The results indicate very clearly that a single cleaning operation only occasionally removes all of the blood, and testing garments, while not uniformly successful will be expected to show results most of the time. Any conclusions from the study will be greatly modified by such factors as the age of the stain before washing, its exposure to heat, light, and similar influences, and biological or chemical effects on the stain after deposition.

THE PHENOLPHTHALIN TEST

It has been noted in this and other laboratories that the performance of the phenolphthalin test is variable. While it showed a sensitivity far greater than any but the luminol test (1) under the conditions used, it does not always yield equally sensitive and definite results when applied to minute amounts of blood on dry filter paper. The same amount of blood in a small volume of solution will often give a much more rapid and sensitive test.

These facts are noted here, not because any remedy for the variability has been found, but because the difficulty was not fully appreciated in earlier work (1). Failure of other workers to find as satisfactory results as claimed can result in distrust of the basic method, when actually the answer will undoubtedly be found in some variation of the method of performing the test. This matter is scheduled for study. It should be noted that the variability casts no doubts on the general utility of the test, since it is definitely a superior one when performed correctly, and any errors that occur will be from false negative rather than false positive reactions.

SUMMARY

The presence of detergents may cause inhibition of agglutination and/or hemolysis when blood spots are grouped.

A comparative study is presented of the effects of different kinds and amounts of laundering on the ability to detect remaining blood.

Some problems of the instability and variations of reagents as they affect blood testing are discussed.

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