An Improved Technique for staining Blood Films with Giemsa Stain

During recent years, the thick-film method of searching for malaria and other blood parasites has largely displaced thin films, especially in what are called 'field surveys' where hundreds and even thousands of blood films may have to be examined. The stain most used is Giemsa (1 c.c. of stain to 15 c.c. of distilled water). Films are stained by immersion for 20 min. in the diluted stain without previous dehæmoglobinization. While this method gives reasonably good results, it has the disadvantage of deeply staining the envelopes of the erythrocytes which adhere to the slide, even though they have lost their hæmoglobin.

If normal saline (0·85 sodium chloride) which has been buffered to *p*H 7·4 is used instead of distilled water, and the films stained for 30 min., staining is greatly improved. The background is clean because the envelopes of all uninfected cells have been removed. On the other hand, the parasitized cells remain, and in *P. vivax* infections Schuffner's stippling is sharply stained and therefore makes species diagnosis easy.

Giemsa stain diluted with normal saline also improves the picture in thin films; it not only reduces the dense appearance of the normal cells but also improves the stippling and shows up the parasites more strikingly. But it is especially for thick films that this improved technique is likely to be most helpful.

Field's technique gives a cleaner background than does Giemsa diluted with distilled water; but by his technique each film has to be stained separately, whereas by the technique quoted above films can be stained in bulk with good results.

The reason for the improvement may be that saline has the effect of dissolving out any globulin and that hæmoglobin in this behaves like a globulin. For solution globulin requires salt solution, and is not soluble in plain water; so it seems possible that in the ordinary way with distilled water more globulin and perhaps even some of the hæmoglobin remains unremoved and so gives a darker staining effect.

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Effect of Crystalloid Solutions prepared in Glass Bottles on Human Red Cells

Various serological techniques employed in blood grouping and allied work involve the washing of human red cells with isotonic solutions, often of saline or sodium citrate.

When carrying out the anti-globulin test[1], it is necessary, after the test cells have been incubated with the serum which may contain a blood-group antibody, to wash them until they have been separated from the serum and are freely suspended in saline. While this washing was being carried out, the cells occasionally became spontaneously agglutinated. This occurrence was observed irregularly, and the strength of the non-specific agglutination was variable. On one occasion, for example, the first washing with an isotonic saline solution produced no agglutination; but subsequent washing produced a very strong and marked agglutination of the red cells. On most other occasions minor agglutination occurred. In the later stages of the test, the washed red cells are mixed with the diluted antiglobulin reagent upon a microscope slide and stirred with either another glass slide or a glass tube; weak false positive results occurred from time to time. Sometimes, when the antiglobulin reagent was being titrated on a tile and stirring of the test cells and diluted reagent was carried out with a small test tube or the tip of a microscope slide, it was observed that weak positive results were obtained in the higher dilutions and in the control test.

Recent techniques have enabled red cells to be frozen solid, and the method of Chaplin and Mollison[2] has been used for enabling test cells, and particularly those of rare blood groups, to be stored so that they can be used whenever required in the laboratory. In order to bring these cells from their suspension in glycerol into a saline solution, they may be treated by washing in successive concentrations of trisodium citrate solution. It was noticed from time to time that non-specific agglutination occurred during the washing of these cells with citrate solutions.

The common factor in all these observations was that agglutination of washed cells occurred only in the absence of serum or when its concentration was very low; trisodium citrate solutions were particularly troublesome.

Extensive investigations were carried out in order to determine the cause of the phenomenon, and it was concluded that the presence of glass was an essential feature. If trisodium citrate solutions are autoclaved in glass containers and afterwards frozen, a solution showing strong agglutination of washed erythrocytes is frequently obtained. If the solution is autoclaved in containers of various different glasses, the strength of the effect varies. If the solutions are autoclaved in plastic bags, no agglutination of the washed cells is observed. Similarly, if the solutions are sterilized by Seitz filtration there is no red-cell agglutination.

A similar agglutination of washed erythrocytes can be brought about by placing the washed cell suspension on a microscope slide, stirring vigorously with a glass rod or the corner of another slide and rocking gently. This phenomenon is also inhibited by serum, but is not always completely inhibited by the low concentration of serum in a dilute antiglobulin reagent. This phenomenon seems to be related to the abrasive action of the two glass surfaces on stirring, and it can be avoided by stirring with a soft plastic rod. This effect is a not infrequent cause of difficulties in the last stage of the antiglobulin test.

It seemed to us, from these experiments, that glass (or some component of glass) was responsible for these phenomena, and following consultations with various chemists and physicists (particularly Mr. D. C. Henry, of the University of Manchester) a solution of colloidal silica was prepared.

It was found that a very dilute solution of silica sol would cause an intense aggregation of normal red cells suspended in saline, but aggregation was absent if the red cells were suspended in serum. Activity was still present at very low concentration of silica, for example, of the order of one part per million. Only certain preparations of silica sol showed the phenomenon, and the factors involved in this are being investigated further.