Heart, lungs and nervous system showed nothing abnormal; the liver and spleen were not enlarged. The urine contained an increased amount of urobilinogen; the stool was normal on microscopic examination. Before the rash appeared the blood was several times examined and found always negative for malarial parasites. Leucocyte count: 15,000 with 78 per cent neutrophils, 20 per cent lymphocytes and 2 per cent large monocytes (10th day).

On the 11th day of illness blood serum separated under aseptic conditions, sealed in sterile ampoules, was sent to Haffkine Institute, Bombay, for carrying out the Weil-Felix test. Dr. S. R. Savoor found the following agglutination titres :—

Proteus OX19 : 1/50 +; 1/125 +; 1/250 +; 1/500 ±; 1/1,250 negative.

Proteus OX2 : 1/50 +; 1/125 negative.

Proteus OXK : 1/50 negative.

Two weeks later, on the 25th day from the onset of illness (28th March, 1946), seven days after the temperature had become normal, the Weil-Felix test was negative (Dr. D. W. Soman, Haffkine Institute).

Treatment was symptomatic, and directed to increase the patient's general resistance by giving high amounts of vitamins, para-amino-benzoic acid being not available here. Recovery was quick, uneventful and complete.

Of the usual vectors, louse and mite can be excluded in this case. The former because of the spotless cleanliness of the patient and his family, the clinical course, the distribution of the rash and the coagglutination of OX2 which hardly is found in louse typhus. The absence of agglutinins for OXK excludes the mite as vector. A high titre for OX19 and a low one for OX2 could be found in flea and in tick typhus.

To decide whether rat fleas or ticks should be incriminated proved here as difficult as in our former investigations (Heilig and Naidu, 1941, 1942). The patient stated that rats were present in his home; our attempts to trap them failed. On the other hand, he was building a dairy farm, kept cattle in his house and frequently went to the grazing places near the city to inspect his cows and buffaloes. I picked some two dozen ticks from 2 cows, kept in his courtyard, and sent them to Haffkine Institute where on 1st April, 1946, two guinea-pigs and 4 mice were inoculated with a ground suspension of 4 ticks; one of the guinea-pigs died on the 19th day after inoculation without showing any pathological signs on post-mortem examination, whereas the other inoculated animals were still alive and apparently healthy on 23rd April, 1946 (Dr. Soman). The other ticks Dr. Savoor took to Kuala Lumpur (F.M.S.) for further investigation.

Summarizing, we have to say that this case of typhus which occurred in Rajputana resembled clinically in every point the cases reported from Mysore (Heilig and Naidu, *loc. cit.*). Bloodshot eyes, character and duration of fever, the well-developed rash which leaves pigmented spots and its distribution all over the body with the exception of the face, good prognosis and speedy recovery—all these points were characteristic also for most of the 32 cases observed in Mysore. Complement fixation tests, performed in three of the Mysore cases, were highly positive for Rocky Mountain spotted fever (Topping, Heilig and Naidu, 1943; Heilig and Naidu, 1944), results almost proving that the Mysore vector is a tick. In the present case, on the other hand, the titre for OX19 was ten times higher than that of OX2; this, taken together with the negative results of animal inoculations with our ticks, may point to the rat flea as the responsible vector rather than to tick-typhus where the agglutinins for the various proteus strains are more equally distributed. But from a single case with only one positive Weil-Felix test definite conclusions cannot be drawn.

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A SIMPLE METHYLENE BLUE-EOSIN SUBSTITUTE FOR LEISHMAN AND GIEMSA STAINS

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The modifications of Romanowsky stain, commonly used in this country, are Leishman and Giemsa. During World War II, these stains became scarce, and were not readily available to workers in India. Intensive work to develop a suitable substitute which could be prepared in the laboratory from materials available in India was undertaken and results of these investigations are presented in this paper.

The Romanowsky stain consists of two parts : the dye and the solvent. The dyes usually employed are methylene blue (or its derivatives) and eosin. When watery solutions of these

dyes are mixed together, dissociation of their respective ions occurs resulting in the formation of new compound or compounds (methylene blue-eosinate), a phenomenon analogous to salt formation. Since this is insoluble in water, it is precipitated. If this precipitate is collected and dissolved in methyl alcohol and is used as a blood stain, it fails to produce the desired Romanowsky effect. If, on the other hand, eosin solution is added to a methylene blue solution previously prepared (a few weeks or months earlier), the precipitate is found to possess adequate staining properties, showing its special affinity for staining the chromatin of the malaria parasite a characteristic red colour, as a result of oxidation of methylene blue. This process can be hastened if a freshly prepared solution of methylene blue is heated in the presence of an alkali. The staining properties are considered to lie not in the methylene blue solution itself but in a new compound called methylene-azure, which is formed as a result of the oxidation of methylene blue.

In the preparation of the new substitute the principles adopted by Wilson (1907), Stévenel (1918), and Krueger and Proescher (1924) who successfully polychromed a solution of methylene blue by using strong oxidizing agents as accelerators have been followed. It was found that not only can polychroming be quickly achieved, but that the formation of azure-eosinate and other compounds responsible for staining are not in any way hampered by the presence of suitable oxidizing agents. In fact a suitable compound giving all the effects of metachromatic staining can be easily and rapidly obtained. Many experiments, using different concentrations of these dyes and oxidizing agents in different proportions of water, were carried out, and the formula finally adopted as the most satisfactory one is given below :—

Methylene blue (medicinal B.P.)	1.0 gm.
Potassium permanganate (medicinal) ..	0.3 gm.
Water-soluble yellow eosin (B.D.H., London) ..	0.4 gm.
Water, distilled or tap ..	250 c.c.

Preparation of the Stain

Divide 250 c.c. water into 3 parts : 100 c.c., 100 c.c. and 50 c.c. Dissolve 1.0 gm. methylene blue in one lot (100 c.c.) of water in a flask, and in another 0.3 gm. potassium permanganate. The water need not necessarily be distilled, for slightly alkaline tap water with a pH value of 7.2 to 7.6 has been found to give satisfactory results.

When the methylene blue is completely dissolved, transfer the solution to a porcelain dish, heating it over the direct flame of a bunsen burner, or a spirit lamp. Allow it to steam but not to boil for about five minutes. Add gradually to this steaming blue solution, the potassium permanganate solution. A fine layer of crystals forms on the surface. Continue heating the mixture for some 10 minutes or so. In the remaining 50 c.c. water, dissolve 0.4 gm. eosin and add it to the steaming methylene blue-permanganate mixture. Mix the contents thoroughly by stirring with a glass rod. A thick yellow scum appears on the surface. Continue heating for an hour or longer till the solution in the dish has evaporated, leaving behind a thick scum with a metallic lustre, at first copper red in colour and when dry green. Care should be taken not to heat the mixture too strongly. The residue in the porcelain dish should be left overnight in a desiccator or in an incubator at 37°C. When completely dry, the scum will peel off as shining green flakes. If necessary, these may be removed by means of a scalpel. Powder the flakes in a dry glass mortar and store the powder thereafter in a dry airtight glass container.

Use of this Powder as a Substitute for Leishman Stain

Take 0.1 gm. of the stain powder, place it in a glass mortar and dissolve it thoroughly by grinding it in 40 c.c. methyl alcohol added in small quantities at a time. The grinding process should be continued till all undissolved particles go into solution. Transfer the contents to a small bottle without filtering. Solutions of the stain made from different batches of powder prepared in these laboratories have retained staining properties for many months, giving excellent results.

The technique of staining blood smears, both thick and thin, is identical with that followed in Leishman's method.

Use of the Powder as a Substitute for Giemsa Stain

The same powder can be used as a substitute for Giemsa stain. Take 0.3 gm. of powder, place it in a glass mortar and grind it adding very gradually a mixture of glycerine and methyl alcohol (25 c.c. each). The freshly prepared solution should be placed in a small glass bottle and allowed to remain at room temperature overnight. On the following day, place the bottle neck deep in a water bath for about 2 hours.

The staining technique in this case is identical with that followed in Giemsa's method and the results achieved have been as good as with any of the standard stains.

Summary

A simple and inexpensive method of preparing a substitute for Leishman and Giemsa stains from dyes readily available in India has been described.

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