

strated in washed and unwashed cells allowed to stand, whether in the clot, in Ringers, in citrate or in normal saline. The author noted, however, that the range from beginning to complete hemolysis was much less in the case of potassium than the other salts. The results with the calcium and magnesium salts were less constant and less reliable than those with the potassium and sodium salts and their readings were less definite in every case in which they were used.

It was attempted to note any change in fragility of red cells when they were incubated in the salt solutions containing 0.0006 molarity of methylene blue under the assumption that the methylene blue would catalyse the absorption of oxygen by the red cells. No change was noted in the methylene blue solutions from that occurring in solutions of the same concentration of salt but containing no methylene blue. The methylene blue solutions were easily read despite the color change.

5511

Influence of Dextrose and of Low Temperatures on Preservation, Transportation and Viability of Malaria Parasites.

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In a study of the factors underlying continued growth and reproduction of malaria parasites (*Plasmodium vivax*) in blood cells removed from the human host, Bass and Johns¹ found that the addition of 0.5% of dextrose to human blood before defibrination apparently prevented an endosmosis into the parasitized corpuscles of toxic substances present in normal serum that quickly destroyed the parasites at body or other temperatures. That by the addition of dextrose the parasites continued their asexual reproduction at a rate directly dependent upon the temperature, up to a maximum of 40°C.

Confronted with the necessity of preserving and transporting blood containing tertian malaria parasites, I have extended these observations on dextrose content and temperature most conducive to prolonging the viability and consequent infectivity of such parasites.

Blood collected from patients presenting the symptoms of thera-

¹ Bass, C. C., and Johns, F. M., *J. Exp. Med.*, 1912, **16**, 567.

peutic "inoculation" malaria was first studied daily in stained preparations of defibrinated blood containing 0.5% to 2% of previously added dextrose, and kept at temperatures of -5 to $+15^{\circ}\text{C}$. Two such experiments conclusively revealed the longest survival of parasites, as shown by normally stained chromatin and protoplasm, in bloods containing 1% of dextrose and when kept at exactly 0°C . In both instances apparently viable parasites could be demonstrated with ease until the tenth day. Dead and dying parasites, as shown by their staining reactions, were most numerous in the higher temperatures with the passage of each 24 hour period. At -5°C . the parasitized cells began to disintegrate within a few hours. All of the parasites in these experiments were schizonts.

The routine method adopted was as follows: Blood was withdrawn from a patient (presenting tertian malaria parasites) with a dry-sterilized all-glass syringe and immediately run into sterile test tubes marked for 10 cc. and containing 0.2 cc. of a 50% sterile solution of dextrose. Defibrination was accomplished by stirring for 10 minutes with a roughened glass rod. The blood was then transferred to small mouth, rubber-stoppered vaccine bottles of 5 cc. capacity and stored in crushed ice. Transportation over long distances has been accomplished by packing in thermos bottles filled with crushed ice plus ice water.

During the past 4 years I have records of 91 inoculations of blood removed from the patient more than 24 hours. These may be summarized as follows:

TABLE I.

0°C .	No. of inoculations	Infection	Non-infection
1- 4 days	67	58	9
5- 8 "	11	7	4
9-12 "	5	1	4
13-15 "	3	1	2
16-18 " *	2	1	1
18-20 " *	3	0	3

* In none of these 5 instances could well stained parasites be demonstrated in stained smears.

Infections have been obtained with bloods shipped by air mail from New Orleans to Los Angeles, or to Jacksonville, Florida; also by combined air mail and train to Denver via Chicago and Billings, Montana. In this latter instance there was an elapsed time of over 5 days.

Inoculations were mostly made on institutionalized patients not subject to probable natural infection.

While control figures are not available on the number of natural immunes present in this community, my impression is that the infectivity of such preserved blood up to 8 days is fully equal to direct transfer of blood from patient to patient.

I wish to thank Dr. H. R. Unsworth, Psychiatrist of the DePaul Sanitarium of New Orleans, for his co-operation by making available the majority of the patients used.

5512

Photodynamic Action of Certain Dyes on the Inactivation of
Staphylococcus Bacteriophage.

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In previous notes the inactivation of staphylococcus bacteriophage by methylene blue (Schultz and Krueger¹) and by toluidine blue (Clifton and Lawler²) was reported.

Various samples of methylene blue in concentrations of 0.1 and 0.01% were mixed with antistaphylococcus bacteriophage, exposed to sunlight for different intervals of time, incubated for 24 hours at 37°C. and then tested for lytic activity. No inactivation was noted when the dye and 'phage were mixed, incubated, and tested in the dark. However, an exposure of 5 minutes, or even less than this time, to indirect sunlight before incubation resulted in complete inactivation of the bacteriophage.

When the methylene blue was added to the bacteriophage suspension in an atmosphere of nitrogen, or in a tube from which the air had been evacuated, no inactivation was noted. This was also true after 30 minutes exposure to direct sunlight and subsequent incubation of these oxygen-free systems. The leucobase of methylene blue likewise did not inactivate this bacteriophage.

Several indophenols having a higher oxidation potential than methylene blue were also tested. They are not photosensitive, did not inactivate the bacteriophage, and were reduced by other oxidizable substances present in the broth.

¹ Schultz, E. W., and Krueger, A. P., *PROC. SOC. EXP. BIOL. AND MED.*, 1928, **26**, 101.

² Clifton, C. E., and Lawler, T. G., *PROC. SOC. EXP. BIOL. AND MED.*, 1930, **27**, 1041.