CRYOPRESERVATION OF THE BLOOD STAGES OF
PLASMODIUM FALCIPARUM AND PLASMODIUM VIVAX FOR
IN VIVO STUDIES*

RICHARD N. ROSSAN
Gorgas Memorial Laboratory, Box 935, APO Miami 34002

Abstract. Cryopreservation in Alsevers-glycerol of the blood stages of Plasmodium falciparum (5 strains) and P. vivax (2 strains) indicated that parasite infectivity for Aotus was retained for more than 1,100 days.

The need for a reliable and reproducible method of cryopreservation of malaria parasites, especially Plasmodium falciparum, has been noted. Various procedures have been reported for the cryopreservation of P. falciparum-infected blood, either with no preservative, the addition of glycerol, or dimethylsulfoxide. The only report using Alsevers solution with glycerol (10%) and low temperature preservation referred to P. berghei.

The present report summarizes our experience using Alsevers solution and 7.5% glycerol for the cryopreservation of various strains of P. falciparum and P. vivax.

MATERIALS AND METHODS

Both donor monkeys, from which parasites were obtained for cryopreservation, and the recipients were Aotus trivirgatus captured on the Isthmus of Panama.

The preservative medium consisted of 9 parts Alsevers solution to 1 part glycerol; 1.5 ml of this mixture was transferred to 5-ml Vacutainer® tube, autoclaved and stored at 5°C. Citrated blood (0.5 ml) from a donor monkey was added to each tube, and the mixture was immediately shell frozen in a dry ice and alcohol slurry. The tubes were stored in a mechanical freezer at -70°C.

The cryopreserved material was thawed rapidly in cold running tap water and inoculated intraperitoneally into a monkey.

RESULTS

Five cryopreserved strains of P. falciparum, and 2 strains of P. vivax (Table 1) were inoculated into Aotus. Parasites of the Vietnam Oak Knoll strain of P. falciparum remained viable after 1,105 days of cryopreservation, while Cheson P. vivax parasites retained viability for as long as 1,123 days of cryopreservation.

Prepatent periods varied from 2 to 28 days in the inoculated monkeys; there was no relationship between the parasitemia at the time of cryopreservation and the length of the prepatent period.

A sample of P. falciparum (Malayan Camp strain) that containing 30,000 parasites/cmm and cryopreserved for 346 days had a sufficient number of viable parasites to infect an Aotus. As few as 10,000 parasites/cmm, when cryopreserved, of P. vivax (Achiote strain) remained infective after 181 days.

Two failures to recover viable P. falciparum parasites occurred: a sample of the Vietnam Smith strain containing 206,000 parasites/cmm that had been cryopreserved for 322 days and a sample of the Panama H strain, that contained 101,000 parasites/cmm and had been cryopreserved for 149 days.

DISCUSSION

Our trials were successful with P. falciparum (Vietnam Oak Knoll strain) parasites cryopreserved for 1,105 days and P. vivax (Chesson

Accepted 3 August 1984.

* This study was supported in part by U.S. Army Medical Research and Development Command Contracts DADA 17-69-C-9126, DADA 17-72-C-2031, DAMD 17-76-C-6069, DAMD 17-82-C-2186, and DAMD 17-83-C-3232. This paper has been designated as Contribution Number 1731 to the Army Research Program on antiparasitic drugs.
Table 1
Positive revival of Plasmodium falciparum and P. vivax strains after cryopreservation in Alsevers-glycerol

<table>
<thead>
<tr>
<th>Malaria strain</th>
<th>No. of Aotus inoculated</th>
<th>No. of days cryopreserved mean (range)</th>
<th>Parasitemia per cmm $\times 10^8$ mean (range)</th>
<th>Prepatent period-days mean (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmodium falciparum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uganda Palo Alto</td>
<td>10</td>
<td>339 (83–725)</td>
<td>1,061 (498–1,971)</td>
<td>7 (2–15)</td>
</tr>
<tr>
<td>Vietnam Oak Knoll</td>
<td>6</td>
<td>467 (96–1,105)</td>
<td>1,377 (63–1,952)</td>
<td>6.2 (6–7)</td>
</tr>
<tr>
<td>Panama II</td>
<td>4</td>
<td>121 (61–299)</td>
<td>996 (773–1,157)</td>
<td>17 (8–28)</td>
</tr>
<tr>
<td>Malayan Camp</td>
<td>3</td>
<td>337 (6–660)</td>
<td>1,268 (30–236)</td>
<td>10.7 (2–17)</td>
</tr>
<tr>
<td>Vietnam Smith</td>
<td>2</td>
<td>492 (433–551)</td>
<td>443 (175–711)</td>
<td>10 (5–15)</td>
</tr>
<tr>
<td>Achiote</td>
<td>3</td>
<td>104 (7–181)</td>
<td>30 (10–70)</td>
<td>15 (8–22)</td>
</tr>
<tr>
<td>Chesson New Guinea</td>
<td>2</td>
<td>691 (222–1,123)</td>
<td>83 (82–84)</td>
<td>5.7 (2–8)</td>
</tr>
</tbody>
</table>

* At the time of cryopreservation, each sample contained 0.5 ml of infected blood.

strain) cryopreserved for 1,123 days. The Camp strain of *P. falciparum* was reported to retain its viability in a chimpanzee after storage in liquid nitrogen for 968 days, and the McClendon strain of *P. falciparum*, after 1,921 days of storage at −78°C, was infective for man. The Chesson strain of *P. vivax*, cryopreserved in liquid nitrogen for 2,860 days, was infective for an Aotus.

The highly reliable technique of using Alsevers-glycerol (7.5%) to cryopreserve *P. falciparum* and *P. vivax* parasites for in vivo utilization was shown by the fact that failures to induce a patent parasitemia occurred in only 2 of 34 attempts. Since thawed samples were inoculated intraperitoneally, deglycerolization was not required as it would have been if the intravenous route of inoculation were used. No apparent adverse reaction in Aotus was observed from the glycerine.

Citrated blood from a donor monkey, infected with *P. falciparum* or *P. vivax*, adapted to Panamanian Aotus, has initiated patent infections in all inoculation trials (more than 800).

References