

Viability of Plasmodium falciparum Frozen in Liquid Nitrogen in the Presence of Dimethyl Sulfoxide.

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**OBJECTIVE:** To determine a simple and reliable method for long term preservation of plasmodium parasitized erythrocytes for both in vitro and in vivo uses.

**DESCRIPTION:** The in vitro culture technique<sup>1</sup> developed in this laboratory offers an extensive approach to further investigation of parasite metabolism and response to drugs. The source of intact parasites for these studies is limited to fresh blood specimens from patients and is seasonally dependent. The glycerol preservation of parasitized cells which has been recommended<sup>2</sup> was found inapplicable for in vitro cultures. Several different attempts have been made to preserve and retain the viability of intact parasites with least hemolysis upon thawing after long-term storage. Different concentrations of dimethyl sulfoxide were added to washed parasitized cells to determine the optimum protection against damage due to freezing and thawing. A similar technique was used to preserve and store simian and rodent malaria for in vivo studies. Viability of frozen P. falciparum infected cells was studied in vitro by radiochemical and morphological methods.

**PROGRESS:** fresh blood specimens were obtained from patients admitted to Cholburi and Somdej Sri Racha Hospitals and kept at 4°C before being processed. A control in vitro culture was made on an aliquot of each specimen. Heparinized blood was washed twice with Tyrode's solution, centrifuged at 1000 rpm in a cold centrifuge. A solution of DMSO in Tyrode's solution was added to washed, packed erythrocytes. Final concentrations of DMSO in cell suspensions were 8, 12 and 15 percent by volume. An aliquot of 0.5-1 ml of cell suspension was transferred into plastic tubing and immediately stored in liquid nitrogen. Frozen cell suspensions were thawed rapidly in a solution of 5% glucose in isotonic saline at 42°C. To obtain a good recovery, an instant thawing process was achieved by agitation of frozen cells in the prewarmed thawing solution. Least hemolysis was seen in specimens containing 12% DMSO. Thawed cells were washed and suspended in culture medium containing C<sup>14</sup>-isoleucine<sup>3</sup>. Growth of parasites in vitro is demonstrable by an increase in the incorporation of C<sup>14</sup>-isoleucine. Morphology of the parasites was studied in serial stained slides made at the same time. (In this study, no foetal cells were included in the culture medium since the parasite growth was studied radiochemically).

Two blood specimens stored in the presence of 12% DMSO for a period of 12 months were studied in vitro. Despite the existence of hemolysis, the remaining intact parasites resumed their viability in vitro and infectivity in vivo. In both specimens, a two-fold increase in parasitized cells was seen after a 72 hr. culture period. (Patient P. from 0.6% to 1.4% and Patient B 0.16% to 0.3%.) The C<sup>14</sup>-isoleucine<sup>3</sup> incorporation rate showed a good correlation with the stages of parasite growth; i.e. radioisotope protein incorporation was observed up to schizogony with no further incorporation being observed until merozoites penetrated new red blood cells. At this time, the incorporation resumes at a rate relative to the increased parasitemia created by repenetration.

Infectivity of frozen simian and rodent malaras was demonstrable by infection in susceptible laboratory animals. A specimen of P. knowlesi, stored for a period of 8 months, was injected into rhesus monkeys

and produced a patent parasitemia on the third day. Similar results were observed with P. barohei in mice.

**SUMMARY:** A simple method for long term preservation of intact plasmodium species is described. P. falciparum infected erythrocytes preserved in the presence of 12% DMSO and kept frozen in liquid nitrogen for a period of 12 months resumed viability in vitro. The infectivity of simian and rodent malaras was also well preserved by this method. Patent infections were demonstrable in susceptible laboratory animals.