حفظ طفيل بلازموديوم برجياى والتوكسوبلازما جونديای بالتبريد العميق

للدكتور محمد الصادق عرفة ، والدكتور نبيل طه نمر ، والدكتورة زبيدة عبد اللطيف كامل ،
والأخصائية مديحة عبد المنعم

الملخص

فى هذه الدراسة أمكن حفظ نوعين من الاوليات التى يعيش أحدها بداخل الخلايا **بلازموديوم برجياى** والآخر خارجها **توكسوبلازما جونديای** باستعمال التبريد العميق ميكانيكيا عند درجتى ـ ٢٠° ، ـ ٧٠° مئوية • وقد أمكن الحفاظ على هذه الطفليات حية بطريقتى التبريد السريع والبطىء باستعمال الجلسيرول ودايمثل سلفوكسيد كمواد حافظة بتركيزات مختلفة • ومن هذا البحث أمكن استخلاص الحقائق الآتية :

أولا : أن التبريد السريع هو الطريقة المثلى لحفظ هذين الطفيلين حيين •

ثانيا : أن الجلسيرول أكثر فائدة فى حفظ الطفيليات التى تعيش داخل الخلايا عن الدايمثيل سلفوكسيد •

ثالثا : أن فترة الحفظ تتناسب طرديا مع تركيز الجلسيرول •

رابعا : أن استعمال محلول الفوسفات المتعادل ومصل العجول الصغيرة أقل كفاءة عن مصل الفئران كمحاليل مخففة للمواد الحافظة

خامسا : أن غسل المادة المعدية بقصد التخلص من الأثر الضار لما يوجد من أجسام مضادة لم يطل فترة حفظ الطفيليات كما كان متوقعا •

Department of Parasitolagy, Faculty of Medicines, Assiut University.
(Head of Dept : **Prof. Dr. M. A.F. Fahmy**)

CRYOPRESERVATION OF PLASMODIUM BERGHEI AND TOXOPLASMA GONDII

(With 2 tables)

By

M.S. Arafa, N.T. Nasr, Zebeida A. Kamel*
and Madiha Abdel-Monem*

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SUMMARY

Preservation of two protozoan parasites, one intracellular *Plasmodium berghei*, and another extracellular *Toxoplasma gonodii* was attempted utilizing the available facilities of mechanical freezing at–20 &–70°C. Viable preservation was accomplished by rapid and slow freezing, using glycerol and dimethyl sulphoxide as protectants in various concentrations. It might be safely concluded that rapid freezing is the method of choice for viable preservation of these tow organisms. Glycerol seems to afford better protection than dimenthly sulphoxide for intracellular parasites. Furthermore the period of preservation is positively correlated with the concentration of glycerol. As diluents for the protectants, phosphate buffer saline and calf serum are likely less efficient than rat serum. Washing of the infective material was intended to abolish the possible harmful effect of coexisting antibodies. However, this step did not seem to enhance the period of preservation.

INTRODUCTION

Much of the efforts undertaken by parasitologists in the past were confined to microscopic observation and morphologic studies. Nowadays however, experimentation on the life cycle, bionomics, physiology, biochemistry, pathology, immunology, etc. is the main goal of parasitology. This goal cannot be achieved without long term maintenance of parasites in the laboratory, either by serial passage in suitable hosts, artificial media or more recently by cryopreservation.

* Research Institute of Medical Entomology, Dokki, Cairo.

The first two methods are agreed upon to be more expensive and laborious, whereas cryopreservation, as indicated by LUMSDEN (1972), offers more than a simple convenient way of storing trypanosomes and probably other parasitic protozoa over longperiods of time. It is more than a simple way of transferring them from one laboratory to another without the need of sending animals. Moreover, it offers conspicuously a way of arresting the continuous reproduction of a population which occurs when it is maintained by serial passage in animals or cultures. Thus, the continuous selection of the reproducing population away from that originally isolated and adapted to the original host, towards one adapted to an abnormal laboratory host is avoided.

The value of glycerolization of the whole blood in viable preservation of *Plasmodium berghei* in the frozen state was proved by JEFFERY (1962). More recently, glycerol and dimethyl sulphoxide (DMSO) were indicated to give good preservation of whole tsetse flies and phlebotomine sandflies, and their trypanosomatid flagellates (MINTER and GEODBLOED, 1970).

So, it was justifiable to test the feasibility of viable preservation of two protozoan parasites, one extracellular *Toxoplasma gondii* and one inracellular *Plasmodium berghei* by deep freezing with the aid of locally available facilities, using glycerol and DMSO.

MATERIIAL AND METHODS

Due to lack of carbon dioxide ice and liquid nitrogen, it was decided to utilize the local facilites available ; namely two mechanical deep freezers —20°C and —70°C.

Viable Preservation of *P. berghei*

Whole infective blood and washed red cells were used for this purpose. White mice infected with *P. berghei* were bled at the peak of parasitaemia, using 2.5% sodium citrate as an anticoagulant.

For washing of the infective cells, the citrated blood was pooled, centrifuged for 10 minutes at 1500 r.p.m. and the supernatant discarded along with the white buffy coat of leucocytes on the top of the deposit. The deposit was washed once in normal saline and recentrifuged for another 10 minutes at 2000 r.p.m. The supernatant was again discarded with the white buffy coat. The deposit was suspended in sufficient amount of physiological saline, to give about 15 infected cells per thin film field.

As protectants, pure glycerol and dimethyl sulphoxide (DMSO) were added in the order of one part and half part of each, to one part of the infective material (whole citrated blood, or washed red cells). Ten percent glycerol in rat serum, calf serum or phosphate buffer (pH 7.7) in the ratio of 3 parts additives to one part infective material were also tested.

The mixtures were then divided into aliquots of 0.5 ml. in serum bottles.

For rapid freezing, the serum bottles were immersed in 95% methanol bath and placed at —20°C for 60 minutes. Thereafter, they were transferred to —70°C and left overnight while still in methanol bath. Next morning they were removed from methanol and stored at —70°C till required.

For slow freezing, the infective material was mixed with 15% and 50% glycerol in phosphate buffer in the ratio of 1 : 1 and 0.5 : 1 and dealt with like for rapid freezing but without resorting to methanol bathnig.

Viable preservation of *T. gondii*

Infective peritoneal exudate of white mice was harvested 3 days after inoculation with the RH strain of *T. gondii.* For preservation experiments, whole exudate and washed organisms were used. Washing of the organisms was implemented as in case of *P. berghei.* The number of organisms in the suspension was adjusted with the aid of a haemocytometer to give a final count amounting to around 6 millions per ml.

As protectants, 10% glycerol and 10% DMSO in rat serum were used. The same steps for rapid freezing using methanol bath as in case of *P. berghei* were followed.

For slow freezing, 10% glycerol in phosphate buffer, rat serum and calf serum were also tested as protectants. As a general rule, 3 parts of the additives were mixed with one part of the infective material. Further steps as in case of *P. berghei* were performed.

To asses the viability of organisms, 0.2 ml. of the mixture after thawing were inoculated intraperitoneally in each of 4 white mice once weekly for a month, then bi-weekly till no more infection was demonstrable throughout an observation period of one year.

In case of *P. berghei,* the infection was demonstarted in blood smears stained with Giemsa stain. 50-100 thin film fields were examined until parasites were found. Blood smears were taken daily.

In case of *T. gondii, the peritoneal* exudate was examined from the third day onwards, using direct smears and stained films.

RESULTS AND DISCUSSIONS

Results are presented in Tables 1 and 2. With successful preservation, infection of white mice with *P. berghei* was usually demonstrable in the peripheral blood with 5 days of inoculation, whereas with *T. gondii,* the peritoneal exudate was found positive within 8 days after inoculation with the test material.

From the foregoing results, it seems that glycerol is superior to DMSO for preservation of *P. berghei,* whereas it appeared of equal value for viable preservation of *T. gondii.*

TABLE 1 : **Viable preservaion of *P. berghei* by deep freezing at -70°C.**

Technique	Additive concentration	Ratio of Additive to infective material	Period of viable preservation in weeks
Rapid Freezing	Pure glycerol	1 : 1	48
	Pure glycerol	0.5 : 1	48
	Pure DMSO	1 : 1	24
	10% glycerol in rat serum . . .	3 : 1	8
	10% glycerol in calf serum . . .	3 : 1	3
	10% glycerol in PBS	3 : 1	3
Slow Freezing	15% glycerol in PBS	1 : 1	3
	50% glycerol in PBS	0.5 : 1	3
	15% glycerol in calf serum	1 : 1	3
	50% glycerol in calf serum . . .	0.5 : 1	3

TABLE 2 : Viable preservation of *T. gondii* by deep freezing at -70°C.

Technique	Additive concentration	Ratio of Additive to infective material	Period of viable preservation in weeks
Rapid Freezing	10% glycerol in rat serum . . .	3 : 1	40
	10% DMSO in rat serum	3 : 1	40
Slow Freezing	10% glycerol in PBS	3 : 1	5
	10% glycerol in rat serum . . .	3 : 1	5
	10% glycerol in calf serum . . .	3 : 1	3

It was also noticeable that high content of glycerol in the mixture gave better results than lower content, as evidenced by more prolonged preservation. Washing of the organisms or infected red cells was intended to abolish the harmful effect of coexisting antibodies. Nevertheless, this step did not enhance the preservation period.

Also it was evident that dilution of glycerol with rat serum gave better results than with calf serum.

As for the rate of freezing, it seems that rapid freezing using methanol bath is much better than slow freezing as it remarkably prolonged the period of viable preservation, under the conditions provided in this study.

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Author's address : Dr. M.S. Arafa, Dept. of Parasit Fac. of Med. Assiut University, Assiut, Egypt.