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A labour-saving method for the in vitro culture of *Plasmodium falciparum*[1]

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Summary

The in vitro cultivation of *Plasmodium falciparum* requires at least daily changes of medium (Trager and Jensen, 1976). Addition of 50 mg per litre of hypoxanthine to medium RPMI 1640 permits to postpone the change of medium for up to 72 hours. A single subculture step is required to remove unlabelled hypoxanthine prior to the use of the cultured material in ^{3}H-hypoxanthine incorporation assays. The method constitutes a considerable saving of time and medium.

Key words: *Plasmodium falciparum;* in vitro; hypoxanthine; postponement of medium change.

Introduction

The original culture technique for *Plasmodium falciparum* (Trager and Jensen, 1976) requires at least daily changes of medium to ensure the good growth of the parasites. This procedure not only leads to operator fatigue – particularly where week-ends are involved – but is also wasteful of medium, containing ten percent of the expensive and rare ingredient, human serum.

Of the simplified systems so far described (Osisanya et al., 1981; Druilhe et al., 1980; Trager, 1980), none seemed to be working satisfactorily in our hands.

The continuous culture of *P. falciparum* (Trager and Jensen, 1976) was achieved in a medium (RPMI 1640) devoid of purines except for those present in human serum. However, since a number of rodent plasmodia (Büngener and

[1] This paper is dedicated to Professor A. Hürlimann, Basel, Switzerland, on the occasion of his 60th birthday.

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Nielsen, 1969; Königk, 1977; Sherman, 1977), as well as *P. falciparum* (Desjardins et al., 1979), readily incorporate radioactively labelled purines, we assumed that the lack of purines in medium RPMI 1640 might be a limiting factor.

Apart from a routine increase of L-glutamine to a total of 5 mmol/l we therefore added 50 mg of hypoxanthine per litre of medium. With no other modifications this allows for normal growth over a 3-day period without medium change.

Materials and Methods

Malaria isolates

Three culture-adapted isolates of *P. falciparum* were used: (i) SGE-1/Senegal, alias "Geneva 13", was a gift from Dr. L. Perrin, WHO, Geneva. The isolate exhibits in vitro resistance to chloroquine. (ii) *P. falciparum* East Africa, originally donated by Dr. W. H. G. Richards, Wellcome Laboratories, Beckenham, Kent, U.K. to Dr. B. Merkli, Roche, Basel, for work in Aotus monkeys. Culture-adapted in this laboratory in August 1980. (iii) FD-III V: a gift from Dr. S. Jepsen, Statens Seruminstitut, Copenhagen. The isolate originates from a case clinically resistant (III) to Fansidar (Black et al., 1981).

All detailed results reported here relate to the first-mentioned isolate; it must be pointed out, however, that the two other isolates behaved in a fully comparable way.

All three isolates were routinely kept in this laboratory in 10 cm diameter Petri dishes containing 10 ml of medium RPMI 1640, supplemented with 10% normal human serum (group A), a total of 5 mmol/l L-glutamine and 50 mg/l neomycine (this medium is hereafter called RPMI). To this were added human group A erythrocytes to a final concentration of 2.5%. Incubation was carried out in an air-tight Modular Incubation Chamber (Flow Laboratories) flushed with a gas mixture of 5% O_2, 5% CO_2 and 90% N_2 at 37° C. For routine purposes the medium was changed daily. This procedure constitutes a minor modification of the original method described by Trager and Jensen (1976).

For experimental purposes the above-mentioned medium was supplemented with 50 mg/l hypoxanthine (hereafter called RPMI/HYPOX).

Procedure

Parasites grown in RPMI were collected by gentle centrifugation (~1000 rpm/min), resuspended to 50% haematocrit and the parasitaemia adjusted to 0.5% or less by the addition of non-infected human erythrocytes. Petri dishes (diameter 10 cm), containing either 10 ml of RPMI or RPMI/HYPOX, each received 500 µl of infected erythrocytes, resulting in a final erythrocyte concentration of 2.5%. RPMI-containing cultures were incubated for three days (Friday to Monday) with daily changes of medium; RPMI/HYPOX-containing cultures were left for the same period without being touched (no renewal of gas phase either).

In view of the fact that we – as well as other laboratories – use cultured parasites in drug-testing experiments (incorporation of ^{3}H-hypoxanthine into drug-treated parasites [Desjardins et al., 1979]), the influence of cold hypoxanthine on the uptake of labelled hypoxanthine was tested as follows: Following the determination of the degree of parasitaemia (thin blood films), both types of cultures were centrifuged, diluted to 2.5% parasitaemia and returned to RPMI. After a 24-h incubation period, parasitaemia was determined once more, adjusted to 1.5% and the suspension diluted to 1.5% haematocrit. Twelve microtiter plate wells were each filled with 200 µl of parasites, originally cultured in RPMI, whereas another 12 received 200 µl of parasites originally cultured in RPMI/HYPOX. The plates were incubated for 24 h, after which all cultures received 20 µl of [G-^{3}H]-

Table 1. Summary of the procedure

Routine procedure	Simplified procedure
Friday: Parasitaemia adjusted to 0.5%; culture medium: RPMI	*Friday:* Parasitaemia adjusted to 0.5% or less; culture medium: RPMI/HYPOX
Saturday: Medium changed	*Saturday:* –
Sunday: Medium changed	*Sunday:* –
Monday: Parasitaemia adjusted to 2.5%; culture medium: RPMI	*Monday:* Parasitaemia adjusted to 2.5%; culture medium: RPMI
Tuesday: Parasitaemia adjusted to 1.5%; haematocrit diluted to 1.5%; culture medium: RPMI; microcultures initiated	*Tuesday:* Parasitaemia adjusted to 1.5%; haematocrit diluted to 1.5%; culture medium: RPMI; microcultures initiated
Wednesday: Addition of ^{3}H-hypoxanthine	*Wednesday:* Addition of ^{3}H-hypoxanthine
Thursday: Cultures terminated and counted	*Thursday:* Cultures terminated and counted

hypoxanthine, 20 μCi/ml in medium. They were then returned to the incubator for an additional 16- to 18-h period.

At the end of the second incubation period the cultures were harvested on an automated cell harvester and prepared for scintillation counting (Insta Gel scintifluor; Packard Tricarb Liquid Scintillation Spectrometer).

Save for the exclusion of antimalarials from the system, this treatment of *P. falciparum* suspensions completely mimicked our routine protocol for the preparation and use of parasites for the in vitro testing of drugs.

Summary of the procedure

See Table 1. Starting material: cultures in RPMI.

Results (Tables 2 to 4)

In experiment 2 a single RPMI/HYPOX culture was left untouched from Friday to Tuesday, with the result that the degree of parasitaemia dropped to 3%. Although the parasites subsequently recovered, the procedure seemed of little value and was, therefore, abandoned (Table 3).

In contrast to the results of the incorporation experiment cited above are the findings of a single experiment, in which microplate cultures were set up with parasites obtained directly from the original RPMI and RPMI/HYPOX cultures without any dilution or subculture in RPMI (Table 4).

Table 2. Degree of parasitaemia

Experiment	RPMI	RPMI/HYPOX
1	12.6%	10.8%
2	13.0%	10.6%
3	6.0%	6.6%

Thin blood films were prepared and the numbers of parasites present in 500 erythrocytes counted (initial parasitaemia: ~0.5%, strain Geneva 13).

Table 3. Incorporation of ^{3}H-hypoxanthine (cpm)

Experiment	RPMI (originally)	RPMI/HYPOX (originally)
1	7 775 ± 474	7 492 ± 183
2	10 508	8 900
3	3 580 ± 196	3 630 ± 150

Table 4

Experiment	RPMI (originally)	RPMI/HYPOX (originally)
1	3 354 ± 138	232 ± 29

Discussion

It is evident from the results that considerable economy in time and medium is achieved by simply adding 50 mg/l of hypoxanthine to the medium. Not only were multiplication rates high and not very different from each other, but the parasites in both types of medium also looked healthy and were microscopically indistinguishable. Moreover, a simple subculture step seems to suffice for the dilution of cold hypoxanthine, which subsequently no longer interferes with the uptake of ^{3}H-hypoxanthine.

The figures so far cited relate to one isolate (Geneva 13) only. However, two additional isolates (East Africa and FD-III V) which were grown using RPMI and RPMI/HYPOX, respectively, gave fully comparable results.

Since all isolates behaved in their usual way on return to RPMI (i.e., approximate doubling of parasitaemia within 24 h; incorporation of ^{3}H-hypoxanthine, etc.), we now use the procedure as a routine technique.

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Black F., Bygbjerg T., Effersøe P., Gomme G., Jepsen S., Axelgaard G.: Fansidar-resistant falciparum malaria acquired in South East Asia. Trans. roy. Soc. trop. Med. Hyg. *75*, 715–716 (1981).

Büngener W., Nielsen G.: Nukleinsäurestoffwechsel bei experimenteller Malaria. III. Einbau von Adenin aus dem Adeninnukleotidpool der Erythrocyten in die Nukleinsäure von Malariaparasiten. Z. Tropenmed. Parasit. *20*, 66–73 (1969).

Desjardins R. E., Canfield C. J., Haynes J. D., Chulay J. D.: Quantitative assessment of antimalarial activity in vitro by a semiautomated microdilution technique. Antimicrob. Agents Chemother. *16*, 710–718 (1979).

Druilhe P., Jacquier P., Lambert D., Gentilini M.: Improvements in the method of culturing *P. falciparum* using umbilical cord serum and medium modifications. Abstr. 10th Int. Congr. trop. Med. and Malaria, Manila 1980, No. 466.

Königk E.: Salvage syntheses and their relationship to nucleic acid metabolism. Bull. Wld Hlth Org. *55*, 249–252 (1977).

Osisanya J. O. S., Gould S., Warhurst D. C.: A simplified culture technique for *Plasmodium falciparum*. Ann. trop. Med. Parasit. *75*, 107–109 (1981).

Sherman I. W.: Amino acid metabolism and protein synthesis in malarial parasites. Bull. Wld Hlth Org. *55*, 265–276 (1977).

Trager W., Jensen J. B.: Human malaria parasites in continuous culture. Science *193*, 673–674 (1976).

Trager W.: Cultivation of erythrocytic stages of malaria. In: The in vitro cultivation of the pathogens of tropical diseases. Tropical Diseases Research Series No. 3, p. 3–13. Schwabe & Co. AG, Basel 1980.