CONTINUOUS CULTURE OF PLASMODIUM FALCIPARUM ASEXUAL STAGES IN "NORMAL" AIR ATMOSPHERE

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Abstract. The growth of six strains of Plasmodium falciparum in 5% CO₂, 5% O₂, 80% N₂ and normal air atmosphere was determined daily by microscopic examination of blood films. All strains were able to grow in flasks without additional gas mixture but significantly lower parasitaemia was observed within the first five days of cultivation. Attempt at cultivating in petri dishes without candle jar technique failed but parasites survived in plasticine sealed dishes. The cultivation in air cannot be recommended for cultures initiated from cryopreserved material or low parasitaemia (0.1–0.3 %) cultures.

Since the first publication of Trager and Jensen (1976) and Haynes et al. (1976) papers the continuous culture of Plasmodium falciparum has become widely used in many malaria laboratories. Various modifications of the original method are in practice but all of them use high carbon dioxide, low oxygen gas mixtures for cultures in flasks and flow systems, or the candle jar technique for dishes. Gas containers and gas mixtures are difficult to obtain in many third world countries especially in field laboratories. The aim of this paper was to find out whether the use of a high carbon dioxide-low oxygen atmosphere for continuous culture of P. falciparum is necessary.

MATERIALS AND METHODS

Parasite: Six parasite lines were kindly made available by Dr. David Walliker.

R33 — originally from Honduras
3DJ — unknown origin, isolated from an airport malaria case in the Netherlands
F32 — from Tanzania
T99, T996 and K1 — from Thailand

Medium: RPMI 1640 (Gibco) supplemented with 25 mmol/l HEPES buffer, 0.2% NaHCO₃, Gentamicin 50 μg/ml and 10% pooled serum was used in all experiments.

Red blood cells (RBC): O type erythrocytes were stored at 4°C in Citrate Phosphate Dextrose Adenin (CPD-A) preservative. Blood was centrifuged (2,000 g/5 min) before use, plasma and buffy coat were discarded. Packed cells were washed twice with RPMI without serum and finally resuspended 1:1 in complete RPMI 1640.

Cryopreservation: Packed infected RBC from culture, preferably with high percentage of ring stages, were mixed at room temperature with an equal volume of 28% glycerol, 3% sorbitol and 0.65% NaCl sterile solution (Rowe et al. 1968). Plastic ampoules containing not more than 0.5 ml of final suspension were rapidly frozen by plunging into the liquid nitrogen.

Thawing: Three-step procedure based on the NIH, USA method was used. 1. The volume of infected RBC, rapidly thawed in a 37°C water bath, was transferred to a centrifuge tube, measured, 12% NaCl solution was added dropwise (200 μl/ml of material) and left for 3 minutes without agitation. 2. 1.6% NaCl solution (10 μl/ml of material) was added dropwise, cells centrifuged and supernatant removed. 3. Solution of 0.9% NaCl + 0.2% dextran (10 μl/ml of material) was again added dropwise, cells centrifuged and resuspended in complete RPMI 1640 for continuous culture.

Synchronisation: Parasites were synchronized by sorbitol lysis (Lambros and Vanderberg 1979)
RESULTS

All results are summarized in Figs. 1—5. The variations in growth of different strains are shown in Table 1. The highest growth rate was found in T966 strain from Thailand (13.7%/5.3%/parasitaemia on day 6), the lowest in HB3 strain from Honduras (7.6%/2.9%/parasitaemia on day 6). These variations being stable in all experimental groups, no other tables were included in this paper.

Table 1. Growth of six tested strains in flasks with 5% CO₂, 5% O₂, 90% N₂ gas mixture and in air. Initial parasitaemia 0.5% (Group 1)

<table>
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<td>1.4</td>
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a — culture diluted to original 0.5% parasitaemia
b — 3D7 strain contaminated on day 5

DISCUSSION

The results show that under certain conditions the asexual stages of *P. falciparum* can be grown using normal atmosphere conditions. However, it also appears that the multiplication of parasites in air is significantly lower, especially at a low parasitaemia. All attempts at cultivating in non-sealed dishes without the candle jar technique failed. The cultivation in flasks which were not tightly closed also failed. It seems evident that the parasite metabolism can alter small isolated volumes of air and thus create an atmosphere suitable for the parasite growth.
at 0.5% parasitaemia (Fig. 1 and Table 1) and least of all in cultures starting with a 2% parasitaemia (Fig. 2).

The experiments with dish cultures were not very successful. Parasites can survive in sealed dishes but the growth rate is very low (Figs. 4, 5). Candle jar technique is simple and dessicators not difficult to obtain. The sealed dish cultures could therefore be only of a very limited use.

Fig. 2. Average growth of six tested strains in flasks with 2% CO₂, 5% O₂, 5% N₂ gas mixture and with normal air atmosphere. Cultures initiated from the cryopreserved material (Group 3). a — culture diluted to original 2.0% parasitaemia.

Fig. 3. Average growth of three tested strains in petri dishes in a candle jar, normal air atmosphere and sealed dishes. Initial parasitaemia 2.0% (Group 5). a — culture diluted to original 2.0% parasitaemia.

Daily changes of medium are not necessary for low parasitaemia cultures in sealed petri dishes and non-gassed flasks. In fact, parasites in culture vessels with 48-hour medium change intervals were growing slightly faster than in cultures with 24-hour changes. Daily maintenance was found necessary in cultures above 5% parasitaemia. Further study is planned to determine the ideal culture conditions in air atmosphere.

Fig. 4. Average growth of three tested strains in petri dishes in a candle jar, normal air atmosphere and sealed dishes. Initial parasitaemia 0.5% (Group 4). a — culture diluted to original 0.5% parasitaemia.

All six strains used in these experiments were stabilized lines well adapted to continuous culture. No fresh isolates were available during experiments. Fresh isolates are notoriously difficult to culture, requiring constant attention. It is therefore improbable that the method described in this paper will be suitable for this purpose. High carbon dioxide-low oxygen atmosphere will always be more productive and well equipped laboratories will find no use for the described technique. It could not be recommended for cultures freshly taken out from deep-freeze storage. However, laboratories lacking the equipment necessary for the maintenance of high CO₂ low O₂ atmosphere could find this method useful.

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КОНТИНУАЛЬНАЯ КУЛЬТУРА АСЕКСУАЛЬНЫХ СТАДИЙ \textit{Plasmodium falciparum} В АТМОСФЕРЕ „НОРМАЛЬНОГО“ ВОЗДУХА

П. Мировский

Резюме. Рост шести штаммов \textit{Plasmodium falciparum} в 5\% CO₂, 5\% O₂, 90\% N₂ и в атмосфере нормального воздуха определяли ежедневно микроскопическим исследованием толстой капли. Все штаммы выросли в бутылках без добавления смеси газов, но наблюдаемая паразитемия была в течение первых пяти дней культивации значительно ниже. Попытки культивировать в чашке Петри без эмикатора (candle jar technique) не удались, но паразиты пережили на запечатанных пластиничатой чашечках. Культивацию на воздухе нельзя рекомендовать для культур с москитной паразитацией (0,1—0,3 \%).

REFERENCES


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