A SIMPLE TECHNIQUE FOR LARGE SCALE *IN VITRO* CULTURE OF *PLASMODIUM FALCIPARUM*

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Abstract. The large scale *in vitro* cultivation method of Fairlamb *et al* (1985) was modified to contain human plasma-supplemented medium in HEPES buffer. After 4 days with no change of medium nor agitation of the culture flask, 27-to 50-fold increase in the starting parasitemia of trophozoites were obtained.

INTRODUCTION

Malaria still poses a threat to the health of residents and travellers in tropical countries. Studies on malaria parasites, in particular *Plasmodium falciparum,* have been greatly facilitated by the advent of an *in vitro* culture system.

The first *in vitro* development of malaria parasites was reported nearly 80 years ago by Bass and Johns (1912). They obtained defibrinated blood from patients infected with *P. falciparum* and cultured the sample at 37°C in a glass vial to which a small amount of glucose had been added. Newly developed rings could be observed after one generation time and only occasionally after one to two additional cycles. Following this report, a number of attempts were made to develop a better culture medium including such methods as short term cultures with Harvard growth medium (Ball *et al,* 1945; Anfinsen *et al,* 1946) and modified Harvard growth medium (Geiman *et al,* 1966). However, only when a new medium, RPMI 1640, developed originally for culture of human leukocytes (Moore *et al,* 1967), became available and was proven to be significantly superior to Harvard medium did it allow for a successful continuous culture of the malaria parasite and which is still being widely used throughout the world (Trager and Jensen, 1976). This technique is particularly suitable for small scale cultures, such as those required for drug sensitivity tests, but it is not adequate for large scale cultures where high yield of parasite is needed, for instance, in parasite enzyme isolation and purification and in studies of parasite organelles. Therefore, other techniques were developed for large scale cultures, such as tilting flask (Jensen *et al,* 1979) and shaken flask (Butcher, 1981) methods. Unfortunately, these procedures require wasteful daily changes of medium either by manual or semi-automated methods. A technique for cultivation of *P. falciparum* without daily medium replacement has been reported which produced high yield of parasites after 3-4 days of cultivation (Fairlamb *et al,* 1985).

In our study, we have simplified the method of Fairlamb *et al* (1985) by replacing human serum with human plasma, changing the buffer system and omitting both flask shaking and adjustment of osmolarity of the medium. By using this simpler procedure, high yield of parasites could still be obtained within a similar period of time.

MATERIALS AND METHODS

Malaria parasite

K1 strain of *Plasmodium falciparum* was used throughout the experiment.

Red blood cells treatment

Human red blood cells (O, Rh^+) from local blood donors were kept at 4°C in blood bags con-

taining citrate-phosphate-dextrose solution. After washing twice with culture medium (see below), packed red blood cells were incubated at 37°C for 1 hour with PIGPA solution in the ratio of 10 : 1. PIGPA solution consisted of 50 mM sodium pyruvate, 50 mM inosine, 100 mM glucose, 500 mM disodium hydrogen phosphate and 5 mM adenine in 0.9% w/v NaCl, pH 7.2. The cells were washed two times with culture medium and the buffy coat removed. The treated red blood cells were resuspended with an equal volume of culture medium and kept at 4°C for further use.

Culture medium

Culture medium was composed of 10.4 g RPMI 1640 (GIBCO), 5.94 g HEPES buffer (Sigma), 2 g glucose, 2.33 g $NaHCO_3$ and 40 mg gentamicin sulfate dissolved in 960 ml distilled water, which was then adjusted to a pH of 7.3. After sterilization through a 0.22 micron GSWP Millipore filter, 850 ml of culture medium were supplemented with 150 ml of serum or plasma to give a complete medium.

Human serum or plasma (O, Rh^+), in 150 ml aliquots, was kept at -20°C, and was thawed at 37°C prior to use.

Synchronization of parasites

P. falciparum culture was synchronized according to the method of Lambros and Vanderberg (1979) as follows. A suspension of parasite cultures was centrifuged at 400*g* at 4°C for 7 minutes and the supernatant liquid was removed. Five volumes of sorbitol (5% w/v) were added to the packed red cells and the solution was mixed thoroughly. The cell suspension was allowed to stand at room temperature for 5 minutes; in this period, cells harboring late trophozoites and schizonts were selectively lysed. After centrifugation at 400*g* at 4°C for 10 minutes, the supernatant solution was removed. The ring-infected red blood cells were cultured for a further period of 24 hours to obtain trophozoite stage for inoculation of the culture flasks.

Procedure of cultivation

Sterile 708 ml screw-capped tissue culture treated flasks (Costar) were used. Each flask contained 100 ml of complete medium with 1% cell suspension at different initial parasitemias. The culture flask was flushed for 1 minute with a gas mixture of 5% CO_2, 5% O_2 and 90% N_2 which was passed through a sterile 0.2 micron Millex-FG gas filter or sterile pasteur pipette packed with cotton plug at a pressure of 15 psi and then the flask was immediately tightly sealed. Culture flasks were placed horizontally in a 37°C incubator for 4 days without change of medium.

Determination of parasitemia

One ml of parasite culture was taken from each flask and centrifuged at 500*g* for 1 min. The packed red cells were smeared and stained with Giemsa. Parasitemia was determined microscopically by counting the number of infected erythrocytes per 5,000 cells.

RESULTS AND DISCUSSION

The technique of Fairlamb *et al* (1985) probably offers the most convenient adaptation for large scale cultivation of *P. falciparum* by users currently employing the Trager-Jensen candle-jar method of cultivation. The modifications to the candle-jar technique introduced by Fairlamb *et al* (1985) involved using treated red blood cells, a change in buffer system, flushing with a gas mixture, shaking of culture flasks and no daily medium replacement. However the method utilizes serum with its concomitant loss of the use of red blood cells and requires shaking throughout the culture period. We have further simplified the procedure by adopting the following changes : replacement of serum with plasma; use of HEPES buffer, which is more readily available and less expensive instead of TES buffer; no adjustment in osmolarity of culture medium; and no agitation of the culture flask.

Cultures were started with 0.5%, 1.0% and 2.0% initial parasitemia, and following four days of cultivation (using plasma-supplemented medium) parasitemias of up to 25%, 33.7% and 54.8%, respectively, were obtained (Table 1). A comparison was made between plasma-supplemented and serum-supplemented medium (Fig 1). After 4 days of incubation, parasitemia in the plasma-supplement medium was 32% whereas serum-supplement medium showed 29%, indica-

Table 1

Percent parasitemia of *Plasmodium falciparum* during 4 days of cultivation with human plasma-supplemented medium. See Materials and Methods for description of the culture conditions; number is mean ± range.

Initial	Day 1	Day 2	Day 3	Day 4
0.5 ± 0.04	0.8 ± 0.08	1.9 ± 0.07	11.5 ± 0.50	25.0 ± 0.50
1.0 ± 0.06	2.3 ± 0.08	4.2 ± 0.16	18.8 ± 0.45	33.7 ± 0.50
2.0 ± 0.08	7.3 ± 0.20	12.7 ± 0.26	39.2 ± 0.75	54.8 ± 0.95

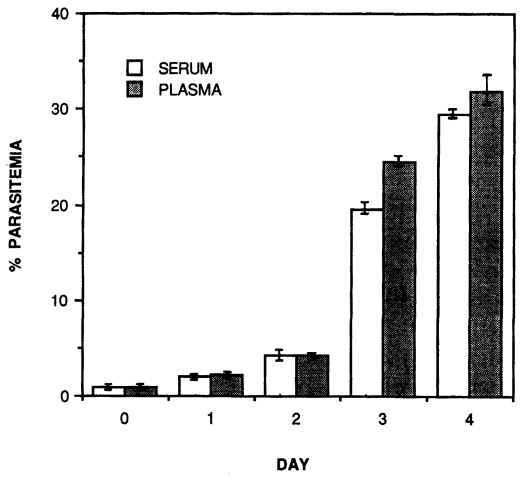

Fig 1—Comparison of percent parasitemia between serum- and plasma-supplemented medium of *Plasmodium falciparum* cultures. See Materials and Methods for details; initial parasitemia was 1%. Bar denotes range of the mean value.

ting no significant difference between using plasma or serum in malaria cultures. Replacement of serum with plasma has been previously reported (Oduola *et al,* 1985).

The procedure described in this report provides a simple, convenient and inexpensive means for large scale cultivation of *P. falciparum* employing facilities already available from the traditional candle-jar cultivation method. Using a starting parasitemia of 2% at trophozoite stage, we have routinely obtained sufficient yield of parasites for studies of DNA replication enzymes (to be published elsewhere).

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