Bulletin of the World Health Organization, 57 (Suppl 1) 17-26 (1979)

Factors affecting the *in vitro* culture of *Plasmodium falciparum* and *Plasmodium knowlesi*

G.A. BUTCHER[1]

Plasmodium falciparum *and* Plasmodium knowlesi *have been established in continuous culture using the basic method of Trager & Jensen. Various parameters of the culture system have been examined, namely, the gas requirements, serum and red cell requirements, frequency of medium replacement, and a comparison of static and agitated cultures made. The most important factors affecting growth* in vitro *seem to be the oxygen tension, red cell concentration, the frequency with which old medium is replaced, and the use of appropriate sera. Preliminary results indicate that horse serum may be possible as a replacement for human serum. Initial studies with* P. knowlesi *indicate that in the course of adapting to culture, parasites may change their antigenic specificity.*

The establishment of *Plasmodium falciparum* in continuous culture (*1*, *2*) and the successful immunization of monkeys against this parasite (*3*, *4*) have given new impetus to hopes for a malaria vaccine. The purpose of the present work was to study some of the factors that may affect growth of *P. falciparum*, particularly with a view to large-scale culture. It became clear from the initial experiments that the use of human serum would impose a considerable limitation on the scale of culture (there is a shortage of human plasma for therapeutic purposes in the United Kingdom) and its replacement by horse serum has been given particular emphasis.

Cultures of *Plasmodium knowlesi* were also established. As more information on the antigenic types of this parasite is available it was hoped this would make it possible to detect any effect of continuous culture on antigenicity. Unfortunately the current shortage of rhesus monkeys and the limitation on supplies of human serum has curtailed this aspect of the work.

MATERIALS AND METHODS

Parasites

Samples of blood from patients with acute infections of *P. falciparum* were frozen by the method of Diggs et al. (*5*) and stored in liquid nitrogen. All isolates came from the Gambia (West Africa) though only one, referred to as HG13, was successfully maintained and used in the work reported here.

Plasmodium knowlesi of the Washington strain (*6*), variant W1, was obtained from rhesus monkeys infected at Guy's Hospital, London.

Basic culture methods

Cultures were set up in Petri dishes (35-mm) containing 1.5 ml of cell suspension or in Corning tissue culture flasks containing 7 or 20 ml volumes. The latter were kept static or shaken in an orbital incubator. Initially all suspensions were 3–4 mm deep (*1*) but agitated flasks were tilted at an angle so that the depth of suspension was variable.

Culture medium consisted of a stock solution of RPMI 1640 (10.4 g in 960 ml of deionized water) kept for up to 1 month at 4 °C, to which other components were added. To 96 ml of the stock solution of RPMI were added:

4.2 ml of 5.5% $NaHCO_3$
0.1 ml of neomycin (10^5 IU/ml)
0.1 ml of heparin (2000 IU/ml)
4.0 ml of 1 mol/l TES (buffered to pH 7.3)[a]

Before the addition of the TES buffer, the medium was gassed with 5% CO_2 to pH 7.4. Heparin was included to prevent small clots appearing in the *P. knowlesi* cultures, but the same stock of medium was used for both parasites. To make the complete medium, serum was added at a final concentration of 100 ml or 200 ml/litre.

For *P. falciparum* cultures, human red cells were used at 10–20% of the total volume unless otherwise

[1] Microbiological Research Establishment, Porton, Salisbury, SP4 0JG, Wiltshire, England. The Microbiological Research Establishment has since been renamed: Centre for Applied Microbiology & Research.

[a] TES = *N*-((tris hydroxymethyl)methyl)-2-amino ethanesulfonic acid.

indicated. With *P. knowlesi*, a 40% volume of rhesus red cells seemed to give better results until the cultures were established, when this was reduced to 20%. Where different culture conditions were compared, starting material came from a common pool of parasitized red cells.

Culture medium was changed twice daily unless otherwise indicated. Spent medium from static cultures of *P. falciparum* with 6% parasitaemias was collected and stored at −20 °C for use in one experiment. The orbital shaker holding agitated flask cultures was automatically switched off 1 hour before the medium was changed; the cells settled to the bottom of the flask and medium could be removed without recourse to centrifugation.

Dishes were gassed in a desiccator and flasks individually from two cylinders—one containing 5% CO_2: 95% air, and one with 5% CO_2: 95% nitrogen. Using rotameters to monitor flow rates, various combinations of oxygen and nitrogen could be obtained, though a mixture of 5% CO_2, 5 or 10% oxygen, remainder nitrogen, was used routinely. Gas was filtered before entering culture vessels. A candle jar (*1*) was used in one experiment.

Cultures were diluted as required with red cells washed with 10 times their volume of phosphate buffered saline (PBS).

Smears made from cultures were stained with Giemsa and up to 2000 erythrocytes were counted per slide to determine the percentage parasitaemia. Only morphologically normal parasites were counted; red cells infected by more than one parasite were counted as single infections. Parasitaemias shown in the figures are usually the mean of duplicate flasks or dishes.

Red cells and sera

In the first few weeks of culture of HG13, and for one later experiment, red cells (AB) collected in heparin or acid-citrate-dextrose (ACD) were used within a few days of collection from donors. Similarly, serum (AB) from clotted blood was also used as soon as possible after collection. Subsequently, group O cells and plasma from "expired" blood (collected in citrate-phosphate-dextrose, and therefore 4 weeks old when obtained), was used routinely. Plasma from the blood packs was incubated for several hours at 37 °C with calcium chloride and thrombin (0.5 ml of 1 mol/litre $CaCl_2$ and 20 units of bovine thrombin per 50 ml of plasma). The serum obtained was stored at −20 °C. The red cells were used up to 1 week after the expiry date.

Horse, lamb, and swine sera from defibrinated blood were absorbed with one-tenth of their volume of human red cells (6 h at 37 °C) and stored at −20 °C.

P. knowlesi was cultured in rhesus serum, or human serum (collected fresh from a donor into a dry blood pack) absorbed overnight at 4 °C with one-tenth of the volume of washed rhesus erythrocytes.

Storage and resuscitation of P. falciparum

As already indicated, infected blood was frozen in liquid nitrogen by the method of Diggs et al. (*5*). However, the material was resuscitated by a slightly simpler modification of that method. The contents of 1-ml ampoules, thawed at room temperature, were mixed with an equal volume of 27% sorbitol (*5*) on a stirrer. PBS was then added slowly (0.3 ml/min) using a syringe pump (Sage Instruments) while mixing; after the first 1.5 ml, a 5-min wait was allowed. More PBS, up to 20 ml, was then added at 0.5–1.0 ml per min.

After centrifugation, the cells were washed once in medium and placed in culture.

Cryohes (hydroxyethyl starch) was also used to preserve deep frozen parasitized blood following the method of Lionetti (*7*) but although the red cells remained intact after thawing, the parasites were not viable.

Determination of variant specificity of cultured P. knowlesi

Variant-specific-schizont-agglutinating (SICA) sera raised in rhesus monkeys as previously described (*6*) were used in a microagglutination test in an attempt to determine the serotype of cultured parasites. Washed cells from 0.2 ml aliquots of culture suspension (made up to 0.2 ml with PBS), were incubated for 2 h at 37 °C with 0.2 ml of a 1/10 dilution of antiserum on a shaker. Thick films were made, stained in Giemsa, and examined for clumps of agglutinated parasites.

RESULTS

P. falciparum: *comparison of different culture conditions*

Of three isolates of Gambian *P. falciparum* resuscitated, only one (HG13) survived beyond 2 weeks in culture. This was subsequently frozen and thawed twice and has since been maintained for 8 months in continuous culture.

The effect of oxygen. A number of different combinations of oxygen, nitrogen, and carbon dioxide have been used in malaria cultures (*1, 2*); although the 5% CO_2: 5 or 10% oxygen routinely used for our cultures seemed successful, this was compared with 5% CO_2 in air and the gas mixture present in a candle jar (*1*) (3% CO_2: 18% O_2: 79% nitrogen). Oxygen at the concentration of 21% is inhibitory to *P. falciparum* (Fig. 1) though the effect takes several days (up to 5 days in another experiment) to become apparent.

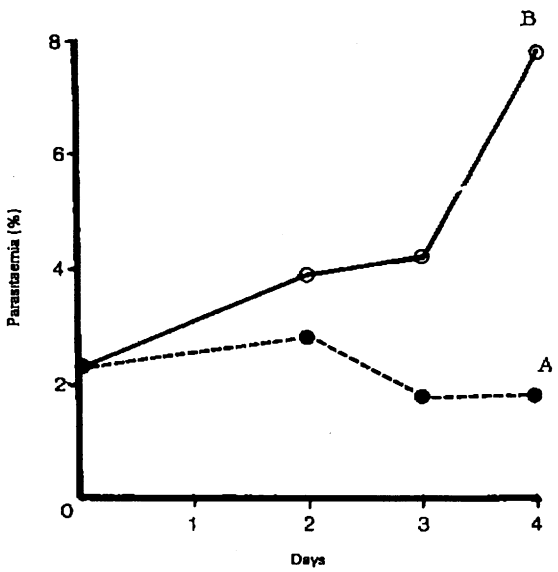

Fig. 1. *P. falciparum*—static, dish cultures; grown in 5% carbon dioxide with (A) 21% oxygen or (B) 5% oxygen.

Concentrations of oxygen between 10% and 21% were not tested (except in the candle jar), but there was no difference in growth rates in 5 and 10% oxygen; less than 5% was inhibitory. There was not any significant difference either in the parasitaemias obtained or the rate of growth between cultures in a candle jar and those with the gas mixture used routinely (Fig. 2).

Red cell requirements. P. falciparum appears to grow equally well in groups AB, A, or O cells, though the latter proved more convenient for our purposes. A comparison of fresh and stored erythrocytes from expired blood was made over a period of 2 weeks. Two "lines" were run in fresh cells from several different donors, and one "line" in expired cells. There was no significant difference in the parasitaemias obtained (Fig. 3).

Cultured human erythrocytes do not appear to deteriorate rapidly *in vitro*; this was shown by two observations. First, red cells preincubated at 37 °C for 2 days before being used to dilute a parasite culture were not much worse at supporting growth than fresh red cells from the same donor held at 4 °C. There was a difference in overall multiplication (3.5 x compared with 5.7 x over 3 days) but the peak parasitaemia was similar for both and at the usual level for the culture conditions (Fig. 4). Second, it was noticed on several occasions that cultures which had reached their peak of 6% after 4 days (the usual peak in static cultures with two changes per day, 10–20% red cells, and

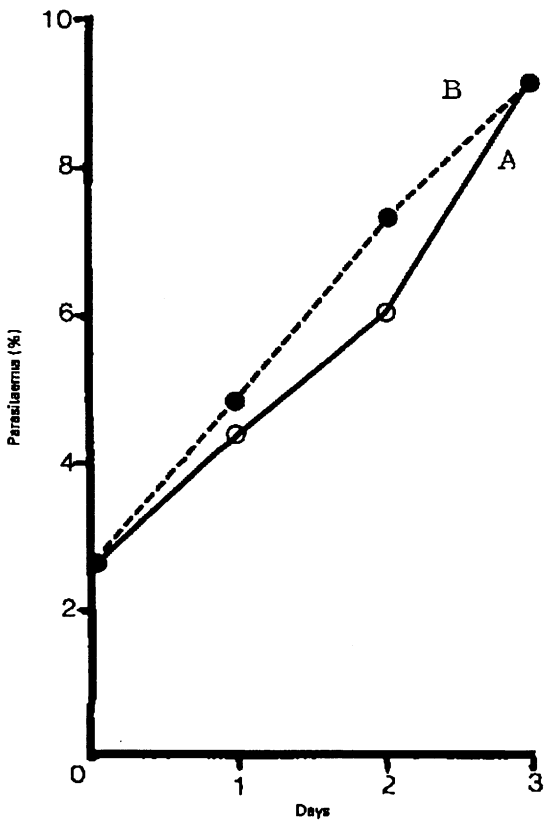

Fig. 2. *P. falciparum*—static, dish cultures; grown in (A) a candle jar (3% carbon dioxide : 18% oxygen : 79% nitrogen), or in (B) 5% carbon dioxide : 10% oxygen : 85% nitrogen.

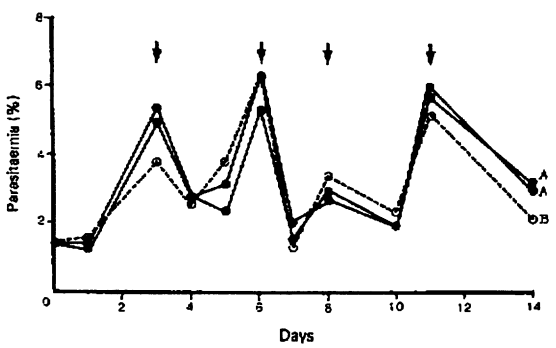

Fig. 3. *P. falciparum*—static, dish cultures; grown in (A) fresh red cells or (B) "expired" red cells. Arrows indicate dilution of cultures with new cells, usually 2–3-fold.

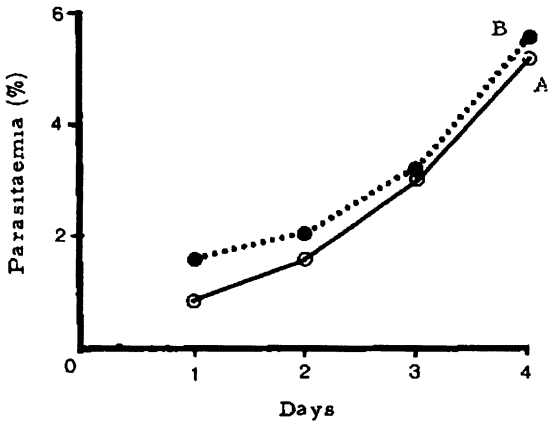

Fig. 4. *P. falciparum*—static, dish cultures; grown in (A) fresh red cells or (B) red cells preincubated for 48 h in usual culture conditions with daily medium change.

using 200 ml/litre of O serum) could be "revived" by reducing the concentration of red cells (Fig. 5 and 9). Parasites would continue to grow in these cells for another 5 days, reaching the same or a slightly higher peak in almost the same time.

The deterioration of medium in vitro. The apparent need to renew the culture medium regularly prompted some investigation of the degree to which the medium became unsuitable. Spent medium that had been used for no longer than 12 h, and which had supported parasitaemias of 6% in the static dishes, was collected over a period and stored at −20 °C. Medium was also collected from cultures of uninfected red cells kept under parallel conditions. Before use, the pH values of both media were checked but required no adjustment. The spent media were used in cultures and compared with new medium. Some of the spent medium was reconstituted with new serum to give 20% by volume of new serum in old medium.

Medium from parasitized cultures did not promote further parasite multiplication (Fig. 6) and the addition of fresh serum did not improve it. Medium from red cell cultures was also inhibitory, giving only half the growth of fresh medium (Fig. 6).

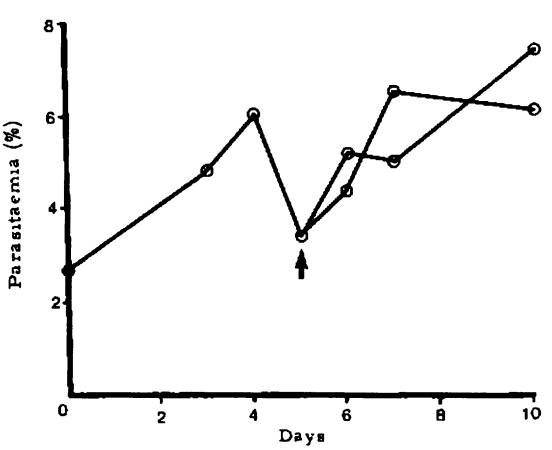

Fig. 5. *P. falciparum*—static, flask culture (7-ml); arrow indicates that the cells were divided between two flasks, reducing the red cell concentration by half (from 10^9 RBC/ml to 5×10^8 RBC/ml).

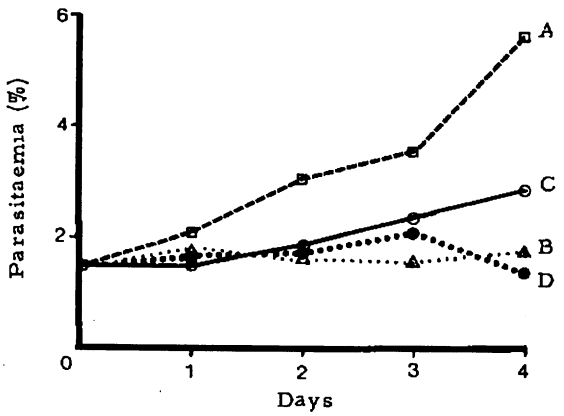

Fig. 6. *P. falciparum*—static, dish cultures; grown in (A) fresh medium, (B) spent parasitized medium, (C) spent medium from cultured red cells, or (D) spent parasite medium supplemented with fresh human serum.

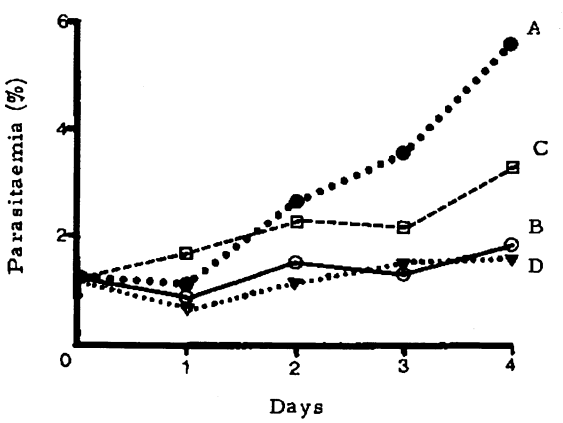

Fig. 7. *P. falciparum*—static, dish cultures but grown in medium with 200 ml of horse serum per litre instead of human serum; (A) in fresh medium, (B) in spent parasitized medium, (C) in spent medium from uninfected red cells, or (D) in spent parasite medium supplemented with fresh horse serum.

A similar result was obtained with cultures with 200 ml of horse serum per litre (Fig. 7) (see below). Change of medium at 8 or 12 h intervals as opposed to once every 24 hours resulted in a significant improvement in parasite growth in human serum (Fig. 8).

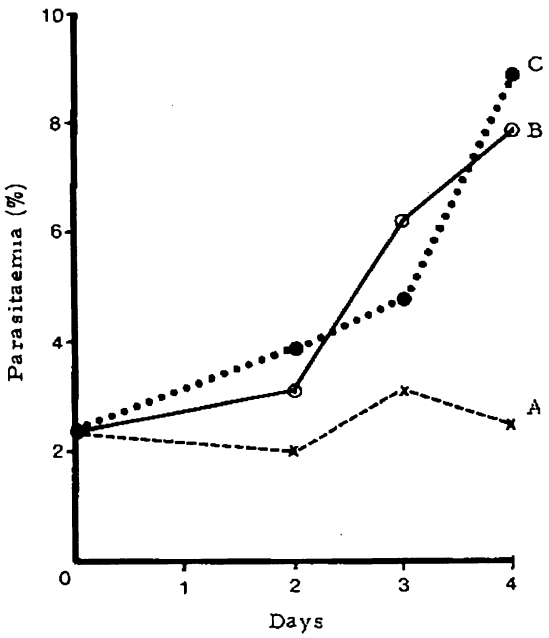

Fig. 8. *P falciparum*—static, dish cultures; medium changed (A) once, (B) twice, or (C) three times during 24 h

Serum requirements. As an alternative to human serum, horse, lamb, and swine sera were tested in culture. These were absorbed with human erythrocytes as described above. Multiplication occurred in lamb serum, and to a slight extent in swine serum, but multiplication in horse serum was definitely superior and a culture "line" was set up in this serum (see below).

Heating human and horse sera for 50 min at 56 °C did not alter their capacity to support growth. A concentration of 200 ml of human serum per litre of medium was more successful than 100 ml/litre, but the reverse was found for horse serum. Routine cultures in horse serum were maintained therefore in a 100 ml/litre concentration (see below).

Static cultures compared with suspension cultures. Previously, the need to use static cultures with the red cells settled at the bottom of a layer of medium 3–4 mm in thickness has been considered important (*1*). Although useful for routine maintenance of the parasite in culture, these static cultures did not give parasitaemias higher than 6–8% (Fig. 5 and 9) with human serum, and twice daily medium changes. Higher parasitaemias for comparable red cell concentrations were

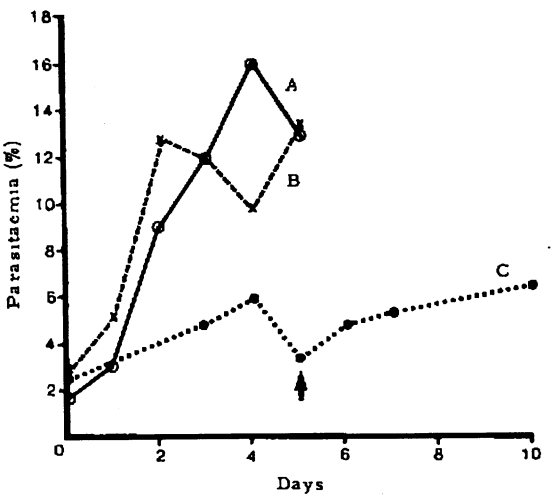

Fig 9. *P. falciparum*—20-ml flask cultures with human serum, shaken, with (A) 3.5 x 10^8 RBC/ml, or (B) 6 x 10^8 RBC/ml. Also (C) one flask held static but divided on day 5 (arrow) reducing the cell count from 10^9 to 5 x 10^5 RBC/ml.

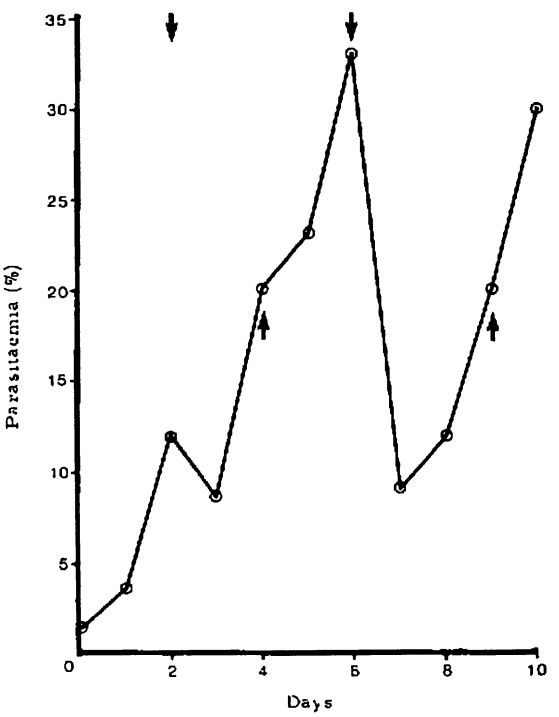

Fig. 10. *P falciparum*—20-ml shaken flasks. Downward arrows indicate dilution several-fold with fresh red cells, upward arrows where the red cell concentration was reduced (from 3.5 x 10^8 to 1.7 x 10^8 RBC/ml).

obtained by agitating the flasks at a rate sufficient to keep the cells in suspension (Fig. 9). By reducing the red cell concentration, even higher parasitaemias were obtained (up to 33% with 1.7 x 10^6 erythrocytes per ml of culture, Fig. 10). This method has been routinely adopted for producing parasites for antigenic analysis, etc.

Growth and maintenance of P. falciparum *in medium containing horse serum.* Initially, *P. falciparum* was maintained at parasitaemias of 1–3% in static cultures with twice daily medium changes, for 1 month. Parasites kept in human serum under similar conditions multiplied at about the same rate, as judged by the total dilution of the cultures over that period (Table 1). Subsequently, a "line" of *P. falciparum* has been maintained in 10% horse serum for nearly 4 months (see below).

Table 1. Culture dilution of *P. falciparum* grown in medium containing human or horse serum

Serum	Weekly dilution factor				Total
	Week 1	Week 2	Week 3	Week 4	
Human	30	48	4	8	4 6 x 10^3
Horse	30	48	4	10	5 76 x 10^4

Maximum parasitaemias of about 4% are obtained in static cultures with two medium changes every 24 h (Fig. 11). More frequent changes gave slightly higher levels, but a single change was likely to lead to a loss of the culture (Fig. 12).

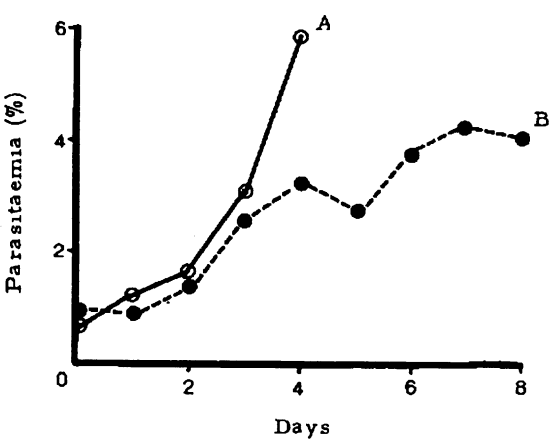

Fig. 11. *P. falciparum*—static, dish cultures in medium with (A) 200 ml of human serum per litre, or (B) 200 ml of horse serum per litre.

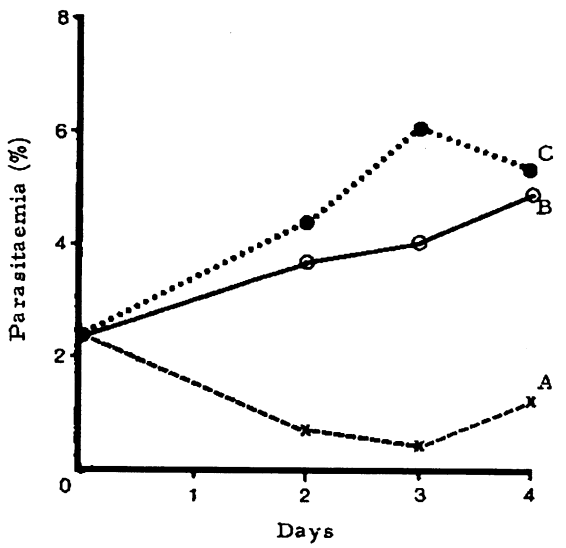

Fig. 12. *P. falciparum*—static, dish cultures; grown in medium with 200 ml of horse serum per litre, medium changed (A) once, (B) twice, or (C) three times in 24 h.

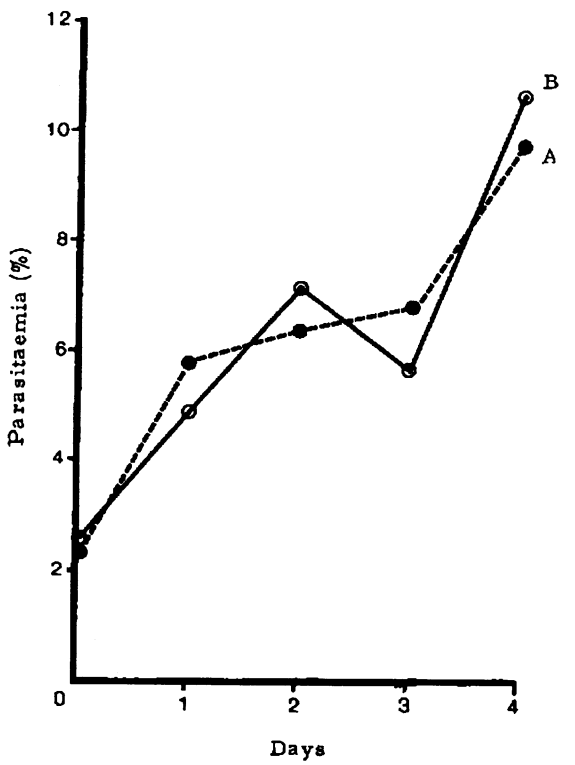

Fig. 13. *P. falciparum*—in medium with 200 ml of horse serum per litre, in (A) static or (B) shaken flasks, at 5 x 10^5 RBC/ml.

Shaking the flasks was no more effective at increasing the maximum parasitaemia than reducing the red cell concentration (Fig. 13). Parasitaemias of about 10% are still the maximum that can be obtained with two daily medium changes.

There was virtually no difference between serum samples from twelve different donors.

P. falciparum: *adaptation to culture*

It is difficult to compare multiplication rates in the early weeks of culture of HG13 with more recent results as initially we used fresh human serum and this is undoubtedly superior to the "outdated" serum used subsequently. However, over the course of 8 months continuous culture in human serum, there has not been any obvious increase in growth rate or in the maximum parasitaemia obtained. Similarly there was not any obvious adaptation to horse serum over 3 months, except that after a single overnight exposure to human serum there was a dramatic drop in parasite levels for 2 weeks and the "line" was nearly lost. This may have been coincidence as it could not be repeated.

P. knowlesi

Because of shortages of serum and monkeys, *P. knowlesi* has not been maintained in culture for more than 1 month. All cultures were started with material from infected monkeys.

Effect of oxygen. P. knowlesi is inhibited by 21% oxygen in the gas phase but not by 5% (Fig. 14). This latter oxygen tension was therefore used routinely.

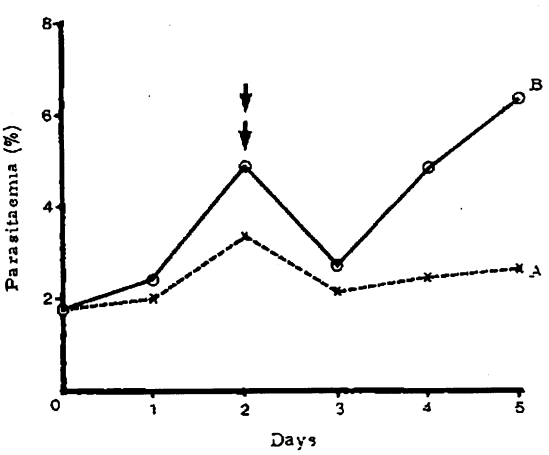

Fig. 14. *P. knowlesi*—static, dish cultures; grown in (A) 21% oxygen, or (B) 5% oxygen. Arrows indicate 2-fold dilution with fresh red cells.

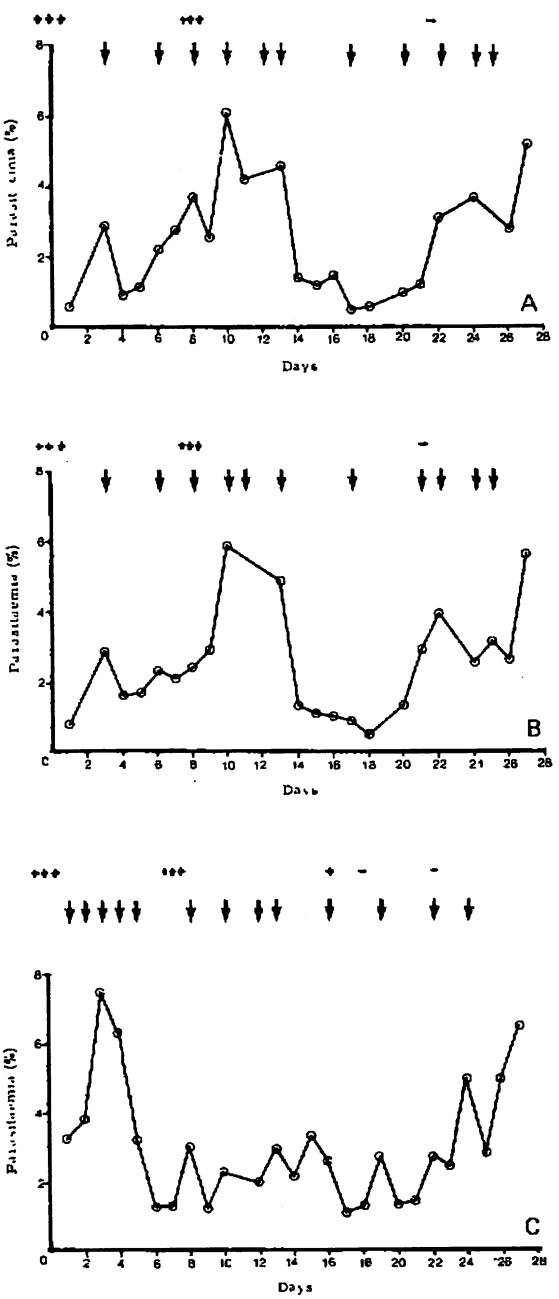

Fig. 15 *P. knowlesi*—parasitaemias over 4 weeks in static dish cultures in three sets of duplicate dishes (A and B from same starting material, C from different monkey donor). + or − indicate degree of agglutination with anti-W1 serum: arrows indicate addition of fresh red cells. (A) run in human serum; (B) run in human serum with 0.1 ml rhesus serum added, (C) run in rhesus serum to day 21.

Serum requirements, red cell requirements, and medium change. Growth was erratic in media containing 200 ml of rhesus serum per litre and some cultures did not survive beyond 1 week. Human serum (group O or AB) collected fresh from a donor bled into a blood pack without anticoagulant was used with greater success. Some samples of human serum had to be absorbed with rhesus erythrocytes to remove agglutinins as described above. Serum from outdated blood was not satisfactory for *P. knowlesi* cultures.

With twice daily medium changes, parasitaemias of 1–5% were maintained, fresh red cells being added 1–5 times weekly (Fig. 15).

Erythrocytes from blood stored in ACD for up to 1 week were as suitable for culture as fresh cells.

Adaptation to culture. P. knowlesi of known variant type (W1) was cultured for 4 weeks; samples were tested weekly with the microagglutination test using agglutinating antisera of known variant specificity. There was no apparent decline in agglutination in the first week but by the end of the third week no parasites of the original antigenic type (W1) could be detected (Fig. 15).

DISCUSSION

It would be interesting to know why some stabilates of *P. falciparum* give rise to successful continuous cultures and others do not. The freezing process may have a damaging effect, particularly as only young parasites survive, but other factors may be important. The high frequency of heterozygous carriers of the sickle-cell gene in West Africa must mean that some parasite donors are of this type, and their erythrocytes may therefore be defective (*8*). Other workers report fewer failures in establishing cultures using parasites from acutely infected Europeans (L. Perrin, personal communication, 1978). Presumably some parasite strains lack the necessary genetic potential to adapt to culture.

Previous workers have demonstrated that high oxygen levels (90%) are inhibitory to a single cycle of parasite growth (*9*) but that the inhibitory effect of 21% oxygen may take several days to become apparent (Fig. 1 and 14). That malaria parasites require at least some oxygen has been known for many years (*9*). There is no demonstrable reason for the use of a candle jar to provide the appropriate atmosphere for cultures (Fig. 2).

We were not able to confirm the finding of Trager & Jensen (*10*) that red cells stored for 22 days in acid-citrate-dextrose or citrate-phosphate-dextrose promoted better growth of *P. falciparum* than fresh cells. In fact, the condition of the red cells in culture does not appear to be one of the major factors limiting parasite multiplication (Fig. 3 and 4) although Trigg et al. (*11*) found that rhesus erythrocytes incubated in culture for up to 48 h were less receptive to merozoites of *P. knowlesi*. However, their cells had been preincubated in medium containing fetal calf serum, which in the writer's experience is not ideal for malaria parasites, and the incubation medium may not have been replaced sufficiently frequently.

A more important limiting factor is the condition of the culture medium, which is "exhausted" not only by the parasites but also by the red cells (Fig. 6 and 7). It is not known how the medium is affected; it is unlikely that the effect is solely due to exhaustion of its buffering capacity (though this may become limiting at high parasitaemias). There was no change in the pH of the spent medium, and experiments in which *P. knowlesi* was cultured in conditions of constantly adjusted pH were not successful in maintaining more than several cycles of growth (H.T. Zwartouw & G.A. Butcher, unpublished observations, 1978). Supplementing spent medium with fresh serum was not sufficient to restore it to its original condition. Thus it is unlikely that this component is simply depleted of its growth promoting properties.

The frequent changes of medium required to obtain higher parasitaemias necessitate large quantities of suitable serum. Attempts to replace serum with synthetic substances in the culture of malaria parasites (*12*) or in other tissue culture systems have rarely been successful, and the easiest solution for producing large quantities of malaria parasites for the development of a vaccine may be to use an animal serum such as that from horses. Horse serum was used almost routinely to culture *P. knowlesi* through a single cycle some years ago (*13*, *14*). Large quantities of serum can be obtained from horses and absorption with human red cells is relatively simple (there is no shortage of outdated human erythrocytes). Although lower concentrations were required with horse serum than with human serum, the medium seems to deteriorate more rapidly in culture (Fig. 7) and needs to be replaced more frequently. Another disadvantage is the low maximum parasitaemia obtained (10%). It is not known what effect continuous culture in horse serum has on the immunogenicity of parasites, or whether it will support other strains in culture. It would also be of interest to know why horse serum is more successful than other sera. It is said to promote more rapid neoplastic transformation and therefore adaptation to continuous culture of lymphocytes than human or fetal calf serum (*15*), but there was no evidence of any adaptation of *P. falciparum* (see below).

While *P. knowlesi* grows well in most rhesus sera, by no means all samples are suitable for culture (*14*).

This may explain why it was necessary to use human serum, though this species, unlike *P. falciparum*, could not be cultured in "outdated" human serum.

The culture method of Trager & Jensen (*1*), using a settled layer of erythrocytes, has been successful in demonstrating that malarial parasites can be maintained *in vitro* continuously, but cultures containing red cells in suspension have given higher parasitaemias in our hands and there is no indication that shaking is deleterious. The concentration of red cells then becomes the limiting factor that determines the quantity of malarial parasites obtained from each culture (Fig. 10).

The differences in culture requirements of *P. falciparum* and *P. knowlesi*, particularly with respect to serum, may result from the faster growth rate of the simian parasite, which completes its erythrocytic development in 24 h. Thus two changes of medium per 24 h for *P. knowlesi* is equivalent to only one for *P. falciparum*. Differences in metabolic rates and permeability of human and rhesus erythrocytes may also be important.

Some organisms, such as *B. pertussis*, show a loss of some antigens when grown *in vitro* continuously and it is important to establish whether this occurs with malaria parasites. Although with *P. falciparum* we could not identify any adaptation to culture that might result in altered immunogenicity, there was a clear change of serotype of the *P. knowlesi* on several occasions. It may be significant that the parasites multiplied less frequently during the third week of culture (when the loss of W1 was noted) (Fig. 15). Recent evidence from research on trypanosomes indicates that antigenic change may happen spontaneously in the absence of antibody (*16*) and that some variant types may outgrow others (*17*). Similar conclusions may apply to malaria parasites and more sensitive techniques than agglutination need to be developed to define the antigenic composition of cultured material (*18*).

This paper has been entirely concerned with the practical problems of growing malaria parasites in culture and whether, once grown, they will be suitable for vaccine production. As more information is now available on the biochemistry of malaria parasites, it should be possible to devise a more economical medium than RPMI, indeed such a medium has been used for short-term culture of *P. knowlesi* (*19*). With this in mind it should be possible to produce considerable quantities of parasite material by observing the conditions outlined above, namely an appropriate oxygen/carbon dioxide mixture for the gas phase, suitable systems for changing the medium, and an unlimited supply of serum (such as horse serum). It should then be possible to obtain suspensions of merozoites for immunization that are virtually free of human serum or red cell components, by isolating free merozoites from the culture medium (*3*).

ACKNOWLEDGEMENTS

This work was supported by the Medical Research Council and the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases. I am indebted to Miss Susan Barron for skilled technical assistance, and to Dr G. H. Mitchell, Guy's Hospital Medical School, for collecting malaria samples in the Gambia. I am also grateful to the volunteer donors and medical staff at the Microbiological Research Establishment, and to the Commanding Officer, Army Blood Supply Depot, Aldershot, for supplying blood samples.

RÉSUMÉ

FACTEURS INFLUANT SUR LA CULTURE *IN VITRO* DE *PLASMODIUM FALCIPARUM* ET DE *PLASMODIUM KNOWLESI*

La culture continue de *Plasmodium falciparum* et de *Plasmodium knowlesi* a été realisée au moyen de la méthode de base mise au point par Trager et Jensen. Divers paramètres affectant la culture ont été examinés, à savoir la composition de l'atmosphère gazeuse, les besoins en sérum et en hématies, et la fréquence de renouvellement du milieu de culture. Une comparaison entre un mode de culture statique ou avec agitation a également été faite.

Les facteurs essentiels pour la croissance *in vitro* semblent être la concentration en oxygène (qui doit se situer entre 5% et 21%), la concentration d'érythrocytes (qui peut être réduite avec de bons résultats), la fréquence avec laquelle le milieu est renouvelé et l'emploi de sérums appropriés (les résultats préliminaires indiquent que le sérum de cheval pourrait remplacer le sérum humain).

Des études initiales portant sur la culture de *P. knowlesi* montrent que l'adaptation des parasites à leurs conditions de culture peut entraîner une modification de leur spécificité antigénique.

REFERENCES

1. TRAGER, W. & JENSEN, J. B. *Science*, **193**: 673-675 (1976).
2. HAYNES, J. D. ET AL. *Nature (London)*, **263**: 767-769 (1976).
3. MITCHELL, G. H. ET AL *Lancet*, **1**: 1335-1338 (1977).
4. SIDDIQUI, W. A. *Science*, **197**: 388-389 (1977).
5. DIGGS, C. L. ET AL. *American journal of tropical medicine and hygiene*, **24**: 760-763 (1975).
6. BUTCHER, G. A. & COHEN, S. *Immunology*, **23**: 503-521 (1972).
7. LIONETTI, F. J. & HUNT, S. M. *Cryobiology*, **12**: 110-118 (1975).
8. FRIEDMAN, M. J. *Proceedings of the National Academy of Sciences*, **75**: 1994-1997 (1978).
9. TRIGG, P. I. *Parasitology*, **59**: 915-924 (1969).
10. JENSEN, J. B. & TRAGER, W. *Journal of parasitology*, **63**: 883-886 (1977).
11. TRIGG, P. I. & SHAKESPEARE, P. G. *Parasitology*, **73**: 149-160 (1976).
12. SIDDIQUI, W. A. ET AL. *Science*, **156**: 1623-1625 (1967).
13. COHEN, S. ET AL *Nature (London)*, **223**: 368-371 (1969).
14. BUTCHER, G. A. & COHEN, S. *Parasitology*, **62**: 309-320 (1971).
15. SANFORD, K. K. ET AL. *Journal of the National Cancer Institute*, **49**: 513-518 (1972).
16. VICKERMAN, K. *Nature (London)*, **273**: 613-617 (1978).
17. MEIRVENNE, N. VAN, ET AL. *Annales de la Société belge de médecine tropicale*, **55**: 1-23 (1975).
18. DOYLE, J. J. *Advances in experimental biology and medicine*, **93**: 31-63 (1977).
19. TRIGG, P. I. & GUTTERIDGE, W. E. *Parasitology*, **62**: 113-123 (1971).