sence d'anticorps palustres dans un sérum rend celui-ci plus ou moins impropre à permettre la croissance *in* [illegible] espérons pouvoir étudier la relation entre le niveau d'immunité naturelle, le titre des anticorps antiplasmodiaux et le titre d'anticorps inhibant la croissance *in vitro* et ainsi, peut-être, d'apprécier les anticorps protecteurs, bien que les mécanismes cellulaires soient probablement les plus importants dans l'immunité anti-plasmodiale.

L'intérêt pharmacologique, enfin, se situe essentiellement au niveau de l'appréciation de la sensibilité des différentes souches de *P. falciparum* aux amino-4-quinoléines.

Ces substances inhibant, probablement par élévation du pH, les enzymes métaboliques de la paroi de la vacuole nutritive bloquent la croissance des jeunes trophozoïtes. Certaines souches semblent avoir adapté leurs enzymes à fonctionner dans une gamme de pH plus étendue, ce qui serait à l'origine des résistances de souche. Il est donc possible *in vitro*, soit par numération différentielle des trophozoïtes et schizontes, soit par appréciation biochimique de l'activité parasitaire dans une culture, de mesurer la dose mini- [illegible]

car il permet, dans une certaine mesure, un paludogramme, plus difficile à effectuer et à interpréter [illegible] rienne.

Nous avons montré récemment que la chimiosensibilité d'une souche n'était pas une valeur fixe et qu'il fallait tenir compte de l'interférence des facteurs d'hôte dans sa mesure à partir du sang du malade. Par contre, après son établissement en culture, la dose de chloroquine inhibitrice de la croissance d'une souche reste stable.

Il faut donc se garder, après avoir considéré les arguments cliniques comme peu fiables dans l'appréciation de résistance de souches, de concéder une valeur absolue à la mesure *in vitro* de la chimiosensibilité à partir de sang du malade. La chimiosensibilité de souche après son passage en hématies et sérum de sujet sain nous semble d'un plus grand intérêt.

Hormis la question des résistances de *P. falciparum* de la chloroquine, l'étude de chimiosensibilité *in vitro* devrait être une méthode d'avenir dans l'étude de nouvelles substances anti-palustres.

Nous conclurons, en remarquant cependant que la culture *in vitro* de *P. falciparum* n'est qu'un modèle [illegible]

Experience with several isolates of continuous *in vitro* culture of *Plasmodium falciparum* with special emphasis on the development of mature gametocytes

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Experience since the fall of 1977 with continuous *in vitro* cultures of *P. falciparum* are briefly reviewed. All cultures were started from cases of *P. falciparum* malaria imported into Holland. 14 Isolates have been established in continuous culture ; one of these (NF 7) has been maintained in culture for about 2 1/2 years. We use plastic petri dishes in a modified CO_2 incubator supplied with a gas mixture, consisting of 3 % O_2, 4 % CO_2 and 93 % N_2.

Many parameters which influence the growth of the asexual stages of the parasite were studied. These included the composition of the culture medium, the age of the red cells used, the storage conditions of red cells, the gas phase and the pH of the cultures. Comments are made especially in view of their relevance for the production of mature sexual stages of the parasites, i.e. the primary objectives of these cultures.

Optimal induction of gametocytogenesis is tempo-

rarily seen for a period of time in adapted cultures of recently established isolates, wherein a rapid production of asexual stages is inhibited by the withholding of the addition of new red cells and especially by creating suboptimal nutritional circumstances, i.e. not refreshing the medium more frequently than once per 24 hours at a stage when the greater number of rapidly dividing asexual parasites are near starvation level and the accumulation of metabolic waste matter is maximal. Optimal gametocyte maturation requires additionally that exposure to the ambient atmospheric conditions should be reduced to a minimum. Otherwise a premature activation and degeneration of gametocytes occurs. This can be achieved by a rapid daily refreshment of the culture medium or by automation procedures which ensure that physiological conditions of the parasites are maintained at all times.

The culture medium : all attempts at replacing the RPMI 1640 with other media such as Dulbecco's Modified Eagles medium and RPMI 1630 were not successful. Addition of human fibroblast medium to the RPMI 1640 gave variable results with regard to parasite growth and gametocytogenesis. Much better growth of asexual parasites could be guaranteed when the RPMI 1640 medium with hepes was kept frozen at — 20° C in aliquots, until use. At that time 10 % human non-inactivated serum is added and also 0,2 % freshly made bicarbonate solution.

The red cells which have to be added to the cultures are prepared from freshly drawn blood, from which the white blood cells are removed by passing over a column of sterilized Whatman CF11 cellulose powder. The red cells are reconstituted with the original citrate-phosphate-dextrose plasma and stored in aliquots at 4° C for not longer than 2-3 weeks.

Red cell suspensions from blood, stored in this way supported parasite growth better than 3 week old erythrocytes. Also both types of blood were stored with the addition of PIGPA, a solution containing pyruvate, inosine, glucose, phosphate and addenine, which can be used to restore a good ATP level in effete red cells. This addition was shown to produce faster rates of growth of the parasites although it failed to improve the peak parasite densities. At that stage of our studies it became apparent that peak densities of asexual parasites are limited by the nature and conditions of the culture medium and not primarily by that of the red cells.

So the composition of the gas phase was studied, of the gas phase was not critical as long as it was below that of the atmosphere. Of primary importance appears however the maintenance of the pH between 7.2 and 7.4. The starting pH of the culture medium could be altered by using half and double the standard amount of 0,2 % bicarbonate. In one experiment cultures containing these three different amounts of bicarbonate were maintained in gas atmospheres of different CO_2 and O_2 composition. The starting pH was lowest i.e. 7.1 in those petri dishes with half the amount bicarbonate and highest, i.e. 7.3 in those containing the double amount. Those dishes with standard bicarbonate had a pH 7.2. When parasite growth was monitored, it was found that a pH 7.1 does not support growth. At pH 7.2 and 7.3 the peak parasite densities were about the same. However this peak was reached one day earlier in those dishes containing standard 0,2 % bicarbonate when they were exposed to 1 % CO_2 in the gas mixture. The reverse occured in a gas mixture containing higher CO_2 levels i.e. 4 %. The pH measured 24 hours after addition of fresh medium tended to be higher with lower CO^2 content.

The continuous monitoring of pH in relation to the production of acid metabolites by the parasites — their amount depends on the number of parasites and the rapidity of their multiplication — and also the amount of bicarbonate and the CO_2 content of the gas mixture appear to be of paramount importance for a better understanding of the *in vitro* growth of *P. falciparum*. Gametocytogenesis was seen in all our isolates of *P. falciparum* that became established in culture, whether the stage was present or not in the original blood sample of the patient. The number of gametocytes seen in culture progressively increased until a peak was reached. Then it rapidly declined and we were not able to restore optimal gametocytogenesis in these cultures again, although gametocytes were seen occasionally from time to time. When kept in continuous *in vitro* culture for prolonged periods, the ability to produce large numbers of gametocytes appears permanently lost. However the potential for producing gametocytes was not lost during storage of adapted stabilates in the cryopreserved state.

The number of gametocytes seen during peak production, the duration of gametocytogenesis and even the sex ratio are probably innate characteristics of each particular isolate. Quoting from personal information I may add that Richard Carter and his colleages were able with their Z strain of *P. falciparum* to induce in slowly growing cultures a development conversing ring stages to gametocytes, approaching only few rings developed into gametocytes.

Prolonged exposures of the parasites to cAMP,

during the period of rapid asexual growth was uniformely lethal to the asexual parasites. However if cAMP was added at a time when the first degenerate parasites appeared in the culture and when also spontaneously some gametocytes appeared in the culture, then it was possible by the addition of this substance to increase the gametocyte production 10 times. This observation needs further confirmation and studies along these lines might add to the clarification of the mechanism of gametocytogenesis. Gametocytogenesis seems to correlate in one way or another with the starvation of the parasites. Gametocytogenesis seems the first step towards an escape of the parasite from an unsuitable environment.

Gametocyte maturation is the final step which is required. By careful maintenance of the cultures we are able now to achieve exflagellation reproducibly in all isolates producing gametocytes.

We have started with the use of these cultures for the induction of sporozoite production in laboratory reared *A. gambiae* mosquitoes. Although once successfully accomplished, we are not yet able to produce *P. falciparum* sporozoites at will. The days seem nearer now that *P. falciparum* sporozoite and gametes can be used in studies on the expression of sporozoite and gamete specific immune responses under field conditions. This seems relevant for a better understanding of anti-malaria immunity in general and it might be useful for seroepidemiological studies on the intensity of malaria transmission. However our efforts are mainly directed towards contributing to the development of anti-malaria vaccines based on the stages.

Interactions entre *Plasmodium* et les antipaludiques

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INTRODUCTION

La résurgence actuelle du paludisme dans le monde — et qui est un reflet non seulement de la dégradation de la situation socio-économique mais aussi des problèmes rencontrés par les opérations de lutte contre le paludisme — a rendu nécessaire de nouvelles recherches pour des produits thérapeutiques contre le paludisme. Des souches de *Plasmodium falciparum* résistantes à la chloroquine, au proguanil et à la pyriméthamine font leur apparition dans une étendue géographique toujours grandissante, et leur présence sur le Continent africain est maintenant confirmée. Il est impératif de rechercher des médicaments appartenant à de nouvelles classes chimiques pouvant remplacer les antipaludiques actuellement utilisés au fur et à mesure que ceux-ci perdent de leur efficacité thérapeutique. Le développement de nouveaux médicaments se fait selon deux approches : la première et la plus large de ces approches est le passage au crible, de façon empirique, de substances pharmaceutiques ; la seconde est une approche rationnelle qui demande une connaissance des voies métaboliques de l'organisme-cible. Jusqu'à présent la plupart des substances antipaludiques ont été découvertes davantage par tâtonnements que par une démarche rationnelle.

LA BIOCHIMIE DE *PLASMODIUM*

Depuis que Laveran (1) (*) a observé pour la première fois l'action de la quinine sur des parasites examinés dans des préparations de sang frais sous lamelle de verre, nous avons compris petit à petit comment certains antipaludiques entravent les processus vitaux des parasites (2). Depuis la Deuxième Guerre mondiale, nous avons beaucoup appris sur la biochimie des stades asexués intraérythrocytaires des parasites du paludisme. Par contre notre connaissance de la biochimie de tous les autres stades de ces organismes obligatoirement intracellulaires reste désespérément pauvre, et ceci est un domaine qui, clairement et de façon urgente, a besoin de recevoir toute notre attention. Nos connaissances actuelles ont été, en grande partie, obtenues à la suite d'essais *in vitro* de culture de plas-

(*) (1 à 26, voir Bibliographie.)