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# NOTA PRÉVIA

## INSULIN REDUCES THE REQUIREMENT FOR SERUM IN *PLASMODIUM FALCIPARUM* CULTURE

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*Insulin added to* Plasmodium falciparum *cultures (0.2 IU/ml) reduced the requirement for human serum from ten to five percent. This represents an obvious advantage by its serum-sparing effect and by reducing the chances of using contaminated serum in cultures. The growth-promoting ability of insulin was observed either in culture-adapted* P. falciparum *or in newly-isolated samples.*

Keywords: *Plasmodium falciparum.* Malaria. Insulin. Culture medium.

The possibility to keep a parasite in culture represents an important step towards a better knowledge of its biology. It also facilitates the study of the action of chemotherapeutic agents and immune factors in vitro, and constitutes a practical way to obtain parasite antigens. With the development by Trager and Jensen in 1976[8] of a simple and effective system, it has been possible to keep *Plasmodium falciparum* in continuous culture. Parasitized erythrocytes are maintained in HEPES-buffered RPMI 1640 medium supplemented with human serum (100ml per liter of medium) for optimal parasite growth. This requirement is an important limitation of the method, since it is not always easy to obtain fresh human serum in the required amount. Also, serum it can carry dangerous pathogens, or antimalarial antibodies and drugs which interfere with the growth of the parasite. The present investigation was carried out in an attempt find a suitable way to replace or reduce the requirement for human serum in *P. falciparum* cultures.

## MATERIALS AND METHODS

*Plasmodium falciparum* isolates: Two *P. falciparum* isolates were used: F 83/2 isolated in 1980 from an infected patient from Ituxi River, State of Amazonas, and kept in continuous culture for twelve months, and F 66/1 newly isolated from a patient from the locality of Embaúba, State of Rondonia.

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Culture conditions: Cultures were initiated by diluting *P. falciparum*-parasitized erythrocytes with non-parasitized O+ outdated erythrocytes from blood donors to a parasitaemia of 0.1%. Erythrocytes were suspended in RPMI 1640 medium containing 25mM of HEPES, 0.2g/l of sodium bicarbonate, 25mg/l of gentamycin, and either 5 or 10% human type O+ serum, or 5% human serum plus bovine crystalline insulin or NPH zinc insulin (manufactured by Eli Lilly and supplied by CEME) at different concentrations. Cultures were kept in 50ml tightly closed culture flasks, gassed with a mixture of 5% CO2 in air, and incubated at 37°C. Culture medium was changed daily till parasitaemia reached 4%, when changes occurred twice a day. Parasitaemia was assessed by examining 200 erythrocytes in Giemsa-stained smears.

## RESULTS

Although insulin was not able to replace human serum in *P. falciparum* cultures, it allowed a reduction to half the amount of serum required for optimum growth of the parasite. From the different concentrations of insulin used (0.05, 0.1, 0.2 and 0.5 IU/ml), the best results were achieved with 0.2 units per milliliter of medium. The increase of the concentration of glucose (2X the amount in the original formulation of the medium) did not enhance the growth rate of the parasite induced by insulin. The effect of crystalline insulin and NPH zinc insulin on parasite growth was comparable.

Our results show that the decrease of the concentration of human serum from ten to five percent caused a statistically significant reduction in the growth rate of plasmodium (Student's "t" test: $p<0.05$). This occurred both with a newly-isolated sample of *P. falciparum* (Table 1), and with a sample kept in continuous culture for twelve months in medium supplemented with 10% human serum (Table 2). The addition of insulin (0.2 IU/ml) to the medium

*Table 1 – Growth rate of a newly-isolated sample of* Plasmodium falciparum *cultured in RPMI 1640 medium with and without crystalline insulin (0.2 IU/ml).*

| *Days* | *% Parasitaemia in medium supplemented with* | | |
|---|---|---|---|
| | *10% serum* | *5% serum* | *5% serum + insulin* |
| 0 | 0.1 | 0.1 | 0.1 |
| 3 | 1.7 | 0.9 | 2.1 |
| 4 | 4.1 | 2.1 | 3.2 |
| 5 | 9.2 | 5.2 | 7.8 |
| 6 | 16.8 | 8.8 | 11.9 |
| 7 | 16.4 | 9.0 | 11.5 |

*Table 2 – Growth rate of a sample of* Plasmodium falciparum *kept for 12 months in continuous culture cultured in RPMI 1640 medium with and without crystalline insulin (0.2 IU/ml).*

| *Days* | *% Parasitaemia in medium supplemented with* | | |
|---|---|---|---|
| | *10% serum* | *5% serum* | *5% serum + insulin* |
| 0 | 0.1 | 0.1 | 0.1 |
| 3 | 1.0 | 0.4 | 1.3 |
| 4 | 6.1 | 3.2 | 6.2 |
| 5 | 12.2 | 7.4 | 11.4 |
| 6 | 16.0 | 6.1 | 13.7 |
| 7 | 17.7 | 8.8 | 17.1 |

containing 5% serum reconstituted its parasite supporting properties, and allowed parasites to multiply at rates comparable to those obtained with medium supplemented with 10% human serum ($p>0.1$).

## DISCUSSION

Insulin has been used as an essential component in several formulations of serum-free media for culturing different mammal cell lines[1,6]. The basis for its growth stimulation property is unknown, although it may be related to its ability to stimulate the incorporation of glucose in both glycogen and fatty acids[5]. We now show that the addition of insulin (0.2 IU/ml) to *P. falciparum* cultures reduces the requirement for human serum to half its usual concentration. This represents an obvious advantage by its serum-sparing effect and by reducing the chances of using contaminated serum in the cultures. Our attempts to replace serum by insulin were not successful, even when medium was supplemented with hypoxanthine and glucose. It is possible that some other growth factors such as transferrin, triiodothyronine, testosterone or hydrocortisone are necessary.

It is interesting to note that both crystalline and NPH zinc insulin attained comparable results as a serum supplement. This suggests that: (a) the stability of insulin does not appear to be critical (in serum-free media 90% of crystalline insulin is destroyed within 1 hour at 37°C[5]), and (b) zinc stabilizer has no growth-inhibitory effect in our system, as it has been shown in cultures of other cells[2].

The best supplement for medium in *P. falciparum* cultures is fresh human serum in concentrations of 100ml per liter[7]. The growth-promoting ability of human serum varies widely from lot to lot, some being able to support optimum growth even at 5% concentrations[4]. This latter effect has also been observed with the same concentration of human serum pooled from 15-20 donors[3]. However, both the selection of suitable lots of human serum and the procurement of pools of sera from several donors are very laborious procedures. Our results show that, by adding insulin to the system, even non-good growth promoting sera, like the one used in our experiment, may be used at 5% concentrations. This effect was observed both with culture-adapted *P. falciparum* and with newly-isolated samples. It would be interesting to know

whether insulin could reduce even more the requirement of a good-growth-promoting lot of human serum.

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## RESUMO

*A adição de insulina (0,2) UI/ml) à cultura de* Plasmodium falciparum *reduziu a necessidade de soro humano de dez para cinco por cento. Isto representa uma óbvia vantagem não só pela economia de soro como pela diminuição do risco de se utilizar amostras de soro contaminadas nas culturas. Esta capacidade da insulina de promover a multiplicação de plasmódio foi observada tanto em relação à* P. falciparum *adaptado à cultura por 12 meses como em amostras recém-isoladas do parasito.*

Palavras-chaves: *Plasmodium falciparum.* Malária. Insulina. Meio de cultura.

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