Ann. Soc. belge Méd. trop.

1978, 59, 89-93

MALARIA CONGENITALE DE LA SOURIS ET ADENOSINE TRIPHOSPHATE (ATP)

par

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Translated from French.

Congenital malaria in mice and adenosine triphosphate (ATP).

In previous research on congenital mouse malaria with *Plasmodium berghei* two of us (Gillet and Herman, 1971 a and b; 1974) observed that the parasite regularly passes the placental filter from the 15th day of gestation toward a fetal environment unfavorable to its development. We have thus been led to raise the existence of a factor braking in the fetus the multiplication of the plasmodium and explaining the rarity of the acute cases of malaria after the birth.

A recent review (Oelshlegel and Brewer, 1975) has focused on a possible relationship between the evolution of plasma erythrocyte forms and the adenosine triphosphate (ATP) content of red blood cells. We took this hypothesis into consideration in the hope of finding an explanation for the phenomenon summarized above. In the present study, ATP was assayed in the blood of fetuses aged 12 to 19 days, mice aged 0 to 20 days and finally 40-day adult mice.

Methods.

Mouse. The mice are of TB breeds whose adults are particularly susceptible to *P. berghei* (Gillet and Herman, 1971b).

Levies. Fertilization of the mouse is verified by demonstration of vaginal keratinization. Fetuses of 12 to 19 days are taken from the thermocautery, carefully avoiding any contamination with maternal blood, and bled in a watch glass, taking blood from the heart in a heparinized tube. The blood of two to six fetuses of the same age is collected in a sample in order to obtain a quantity total blood from 30 to 50 µl.

Mice from 0 to 10 days are bled to the heart. Those of 20 days and adults are bled at the tip of the tail. By age, four to six samples were assayed and the results were analyzed by the classical variance method.

Assays. The hematocrit is measured after centrifugation at 2000 rpm for 5 minutes. The red blood cells are extracted into 0.6 N perchloric acid and then centrifuged for 20 minutes at 2000 rpm. ATP is assayed in an aliquot (20 to 100 µl) of the supernatant by an enzymatic method (Jaworek, Gruber & Bergmeyer, 1970).

Results

Averages of 4 to 6 assays are plotted in Figure 1, curve A, in mg ATP per 100 ml of red blood cells in relation to the age of the sample: fetuses, young mice and adult mice. In the latter, the concentration of ATP is 116 mg per 100 ml of globules, which should be considered as normal. The intra-globular concentration of ATP cannot be calculated accurately since we have not measured the water content in the globules and we will ignore the intracellular distribution of ATP and in particular the proportion of ATP combined. Nevertheless, assuming an average concentration of 65% globular water, and assuming that the amount of ATP bound is negligible, the intraglobular concentration of ATP is estimated to be 3.6 mMoles per liter of intracellular water.

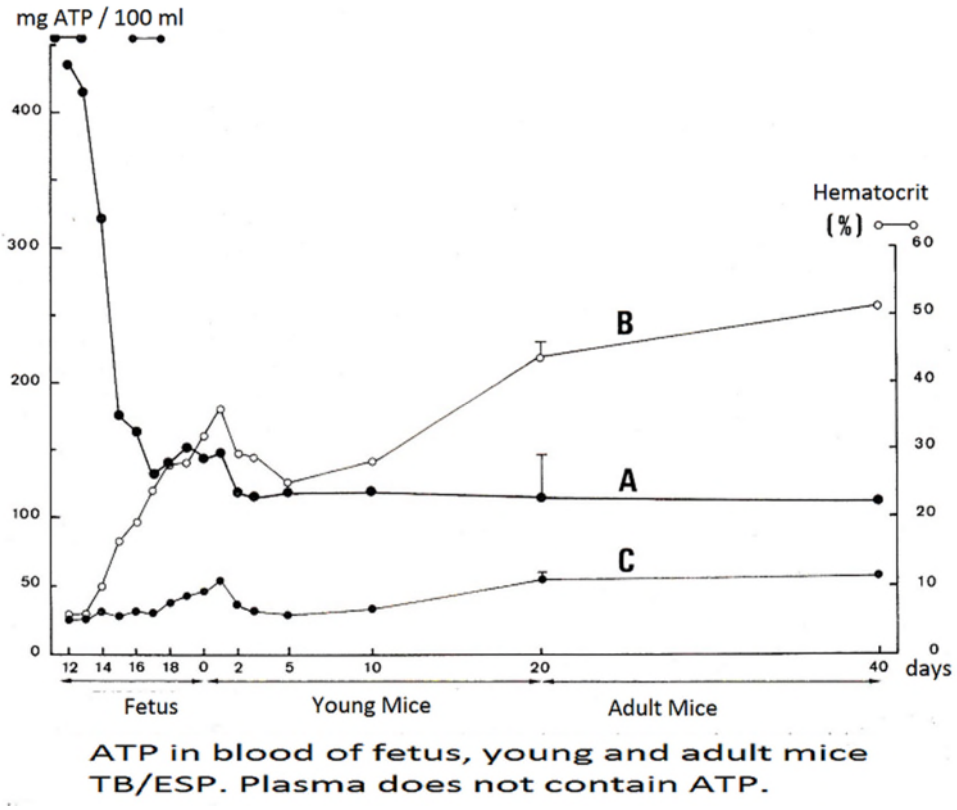

Figure 1

The maximum ATP concentration in the 12-day fetus reached its maximum: 438 mg per 100 ml, a 3.8-fold concentration greater than that measured in adults (116 mg / 100 ml). It is noted that the globular concentration of ATP decreases mainly between the thirteenth day when it reaches 417 mg / 100 ml and the fifteenth day when it is no more than 178 mg / 100 ml; This fall of 239 mg / 100 ml in 48 hours is particularly brutal, as this value is twice as high as the concentration of ATP found in adult mice (116 mg). From the 15th day of gestation to the 3rd day after birth, the curve shows oscillations with little statistical significance. It is possible that the evolution of this curve can be explained by the succession of three populations of erythrocytes described (Fantoni et al., 1969, Marks and Rifkind, 1972): rapid decrease of embryonic erythroblasts from the twelfth to the sixteenth day, Then disappearance of the fetal erythroblasts with a maximum on the eighteenth day and then towards the end of the fetal period, appearance of adult erythrocytes which would reach their normal count 4 to 6 days after the birth.

The hematocrit is shown as a function of age in Figure 1, curve B. It is very low in the twelve-day fetus (6%), and gradually rises during gestation, reaching a maximum of 36% at the end of farrowing. It then diminishes appreciably until the sixth day after birth, when it reaches only 25%. Then it rises towards the value of the adult, 51% on the twentieth day.

Total blood levels of ATP vary in a similar manner to hematocrit (curve C). Separate measurements show that the plasma ATP level is negligible, regardless of the age of the animal. The total blood levels of ATP thus simply equal the products obtained by multiplying the globular concentrations by the hematocrit. They show a temporal evolution very similar to those of the hematocrit (curve B).

Discussion

In an attempt to explain the unfavorable influence of the fetal environment on the evolution of *P. berghei*, we first think of the possible role of fetal hemoglobin, a hypothesis advanced in human malaria (Lehmann, 1953, Allison, 1954; Gilles, 1957). According to a study by Van Ros et al. (1972), this factor would not occur in mice in the observed phenomenon; the same authors excluded a deficiency of glucose-6-phosphate dehydrogenase (G-6-PDH).

According to the hypothesis of Oelshlegel and Brewer (1975), a reduction in the globular ATP concentration would provide some degree of protection against malaria. The arguments in favor of this view are suggestive. A high initial level of ATP in erythrocytes appears to have a significant influence on the rate of development of malaria parasites and on the severity of access with *P. falciparum* in man (Brewer and Powell, 1965) than with *P. cynomolgi* in the monkey (Eaton and Brewer, 1969). Similarly, Brewer and Coan (1969) reported that an acute parasitaemia of *P. berghei* or *P. vinckei* in rats may be accompanied by a reduction in globular ATP up to sixth of the initial level (from 6.67 to 1.12 micromoles of ATP per gram of hemoglobin); Fletcher and colleagues (1970) reported similar phenomenon in monkeys.

These findings are consistent with Brewer's (1967a) genetic study, which finds that the average level of erythrocyte ATP is lower in blacks in the United States of America than in white (respectively 3.17 and 3.47 micromoles of ATP per gram of hemoglobin); The first, originating from Africa, have been exposed for generations to malaria which has selected genes reducing the content of erythrocytes to ATP and conferring to the carriers a certain resistance to the affection. This same hereditary factor would explain the protection against malaria provided by the β-thalassemia gene, whose heterozygous carriers have a globular ATP level which is 16% lower than the normal value (Brewer, 1967b, Brown, 1976) .

The resistance of the fetuses of mice to *P. berghei* cannot be explained by the hypothesis of Oelshlegel and Brewer (1975) since the rate of erythrocyte ATP is 1.5 to 3.8 times higher in the resistant fetus than in the susceptible adult. The factors involved in this phenomenon are still to be found.

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Summary — Adult TB mice are very susceptible to *P. berghei.* The ATP concentration of their erythrocytes is 116 mg/100 ml. The foetus, however are resistant against malaria; the ATP concentration of their erythrocytes is 153 mg/100 ml just before birth, and 438 mg/100 ml at the 12th day of gestation. It is concluded that the resistance of the mice foetus against malaria is not explained by a decreased level of ATP in their erythrocytes.

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Reçu pour publication le 22 novembre 1977.

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