Journal of
Medical Sciences

Serum Steroid Levels in Mice Infected with *Plasmodium berghei berghei*

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Serum levels progesterone and testosterone were measured by radioimmunoassay (RIA) in female and male mice infected with *Plasmodium berghei berghei*, respectively and in uninfected female and uninfected male mice correspondingly. The mean SEM serum progesterone (nmol L^{-1}) of 2.41±0.31 in infected female mice is lower than 3.45±0.44 observed in uninfected mice. The mean SEM serum testosterone (nmol L^{-1}) of 2.24±0.2 in infected male mice is significantly higher than 1.39±0.14 observed in uninfected mice. The results provide evidence to suggest that malaria parasite *Plasmodium berghei berghei* lowers peripheral levels of progesterone in female mice. However, it appears to increase peripheral levels of testosterone in male mice. Malaria is known to be an endemic disease of the tropics. In females, the actions of progesterone as an antagonist to both sodium retaining and potassium losing effect of adrenal steroids could be affected. Raised levels of testosterone in males during infection could possibly reduce the risk of aplastic anaemia in the young approaching puberty.

Key words: Progesterone, testosterone, aplastic anaemia, *Plasmodium berghei berghei*

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INTRODUCTION

Gonadal steroids are the male sex hormones (testosterone, dihydrosterone) and the female oestrogens (oesterone, oestriol and oestrogens-oestradiol 17β) and progesterone[1]. The major gonadal steroids are progesterone, testosterone and oestradiol 17β[2]. They are produced in the testis and ovaries. Androgens and oestrogens are also produced in the adrenals of males and females[3]. Their synthesis in the gonads is controlled by the Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and the anterior puituitary hormones[4]. The secretion of FSH and LH is controlled by the hypothalamic gonadotropin releasing hormones also known as LH/FSH releasing hormone and by a feedback mechanism by the target hormones[5].

All steroids are formed from a common precursor, pregnenolone, derived from cholesterol which is in turn synthesized from acetate. All glands that sythesize steroids are capable of synthesizing cholesterol but the liver is the main site of formation[6]. Testosterone is synthesized in the testis. About two thirds of the secreted testosterone is transported (in plasma bound) to the steroid binding globulin, a beta globulin. In the testis, testosterone is bound to the androgen binding protein secreted by the sertoli cells. Androgens are responsible for the development of the male secondary sex characteristics and promotion of organ growth in many growing animals[2,3,7]. Testosterone and FSH are responsible for the maintenance of spermatogogenesis. Most circulatory testosterone is conjugated in the liver mainly into glucoronides and excreted in urine as 17-oxosteroids. Testosterone is also converted by the postrate, skin and seminal vesicles into more potent dihydrotestosterone[7]. Progesterone is secreted by the corpus luteum and placenta in humans, by the corpus luteum in guinea pigs and cattle, by the adrenals in cattle and by the ovaries in the dog fish, starfish and hen. It is an important intermediate in steroid biosynthesis[2,7].

In early pregnancy, hCG stimulates the corpus luteum to secrete progesterone to support the endometruim. The progesterone promotes secretory changes in the endometrium and so prepares the uterus for implanation of the fertilized ovum[8]. It promotes the development of the alveoli and lobules of the breast, so causing the alveolar cells to proliferate, enlarge and become secretory[9]. In humans, progesterone is converted to pregnanediol which is conjugated with glucoronic acid in the liver and excreted in urine. Women in late pregnancy excrete increased amount of pregnanediol while sheep, cows and goats excrete only trace amounts[2,7].

Malaria, derived from the Italian word "foul air" is a parasitic disease caused by organisms belonging to the family plasmodia commonly referred to as malaria parasites[10,11]. Malaria, one of the most prevalent world wide infections occurs in the tropics, sub-tropics and some parts of the temperate regions. It attacks man and other species of animals[10]. It is a deadly disease which lowers life expectancy and a major cause of infant mortality in highly endemic area[12]. The disease inflicts considerable adverse effect on the organs of its host. Such effects include: enlargement of the spleen[13], variable loss of glycogen in the parenchymal cells of the liver, structural changes such as oxidative phosphorylation and abnormal respiration[14]. Prolonged relapsing malaria in patients may result in chronic cardiac disturbances which may progress to the point of cardiac dialation, ventricular aneurysm and cardiac rupture[15]. One of the most important effects of *Plasmodium falciparum* infection is capillary endothelial damage which cause increased vascular permeability leading to impairment of micro-circulation[16]. It is therefore tempting to speculate that biochemical functions of the organs of the body are affected during malaria infection.

This study was designed to find out if malaria parasites have any noticeable effect on the release of gonadal hormones in mice. Gonadal function in male mice will be assessed by plasma testosterone estimation by radioimmunoassay and female gonadal function assessment will be based on plasma progesterone estimation.

MATERIALS AND METHODS

Malaria parasites: Malaria parasites *Plasmodium berghei berghei* used were obtained from a stock maintained at the central animal house, College of Medicine, University of Ibadan. This was prepared as an inoculum. All the mice were screened for malaria parasites at the beginning of the experiments. All were found to be free of infection.

Experimental mice: Mice used were of the Central Animal House, College of Medicine, University of Ibadan. They were albino of the same strain and bred locally by the staff of the Animal House. The groups of experimental mouse used were the following:

- *Plasmodium berghei berghei* infected mice
- Saline treated mice (controls)
- Untreated mice (controls).

All the mice used were injected intraperitoneally with 0.1 mL inoculum of 1×10^5 *Plasmodium berghei berghei* infected erythrocytes from a donor mouse. Tail blood films were made from each from the fourth day of inoculation. The films were stained with Leishman dye and the degree of parasiteamia assessed by microscope counts. Infected mouse were bled by cardiac puncture.

Sera were collected after 1 h of erythrocyte extraction. Uninfected mice either injected with saline from day or untreated mice (not injected) served as control. Controls were similarly bled and sera collected. Test and control sera were frozen at –20°C and then analysed[17-19].

RIA for progesterone and testosterone: Serum progesterone and serum testosterone were measured by RIA[20,21] using the dextran-coated charcoal method according to the technique of Thornecycroft and Stone[22].

RESULTS AND DISCUSSION

The results showed a decrease in values of serum progesterone in mice infected with *Plasmodium berghei berghei* (Table 1).

There is a significant increase in values of serum testosterone in mice infected with *Plasmodium berghei berghei* (Table 2).

The observed reduction in the mean±SEM serum progesterone in female mice infected with *Plasmodium berghei berghei* compared to uninfected female mice confirm that malaria parasites tend to decrease serum progesterone levels in female mice. Since the measured serum progesterone represents a contribution from adrenals and ovaries among other steroid producing glands, it cannot be said categorically at this stage that the parasites are inhibiting ovarian function. Although it may be misleading to ascribe the observed lowering effect to any particular one of these sites of progesterone production, it is speculated that more of the effect will be at the ovarian level. The effect may be due to the parasite per se or pyrexia caused by the malaria fever. One expects serum progesterone to be increased by pyrexia but in this study, it appears not to be so. It may be that steroid synthesizing enzymes are inhibited by malaria parasites in female mice. If the rate of inhibition is more than the induced increase by pyrexia, the overall effect would be a decrease. The low serum progesterone levels observed in the saline-treated group of mice may be due to haemodilution.

The mean±SEM serum testosterone levels was observed to be significantly higher in male mice infected with *Plasmosmodium berghei berghei* than in uninfected mice. Saline treatment had no effect on the mice. It therefore appears that malaria parasites were responsible for the increased peripheral level of testosterone in mice. These observed results suggest that the pyrexia (as in malaria fever) may have impaired steroid conjugation by hepatic cells. This might have decreased steroid excretion, thereby increasing serum levels. It can also be postulated that malaria parasites might have enhanced the conversion of progesterone to its various metabolic products, testosterone being one of them. In

Table 1: Serum progesterone levels (nmol L⁻¹) in infected mice (a), saline treated mice (b) and untreated mice (c). Serum progesterone levels (nmol L⁻¹)

	a	b	c
	3.60	0.10	3.10
	3.20	1.40	4.40
	1.20	1.00	5.20
	2.70	1.50	4.80
	1.50	2.50	2.20
	3.20	2.40	1.90
	1.90	1.30	2.50
	2.00	0.70	3.50
N	8.00	8.00	8.00
Mean	2.41	1.36	3.45
SD	0.83	0.80	1.25
SE	0.31	0.29	0.44

Comparison of values of progesterone in the groups of mice (p-values)
When a is compared with b, p<0.05, When a is compared with c, p>0.05

Table 2: Serum testosterone levels (nmol L⁻¹) in infected mice (a), saline treated mice (b) and untreated mice (c). Serum testrosteone levels (nmol L⁻¹)

	a	b	c
	2.00	1.92	1.60
	1.76	1.52	0.96
	2.32	0.72	1.60
	2.88	1.68	0.88
	3.28	2.32	1.68
	1.92	2.24	0.96
	1.77	2.32	1.68
	2.00	1.68	1.76
N	8.00	8.00	8.00
Mean	2.24	1.80	1.39
SD	0.60	0.50	0.34
SE	0.20	0.19	0.14

Comparison of values of testosterone in the groups of mice (p values)
When a is compared with b, p>0.05, When a is compared with c, p<0.05

such a situation, one would observe low serum progesterone levels with corresponding increase in serum testosterone levels.

Although progesterone has no effect on the kidneys in Addison's disease, its actions as an antagonist to both sodium retaining and potassium losing effect of adrenal steroids could be affected during infection. Testosterone is known to stimulate growth in children and sometimes found to be effective in treating anaemia associated with carcinoma of breast, ureamia, myelofibrosis and chronic myeloid anaemia in children approaching puberty[22]. Similar to those in man, these effects are therefore expected during malaria infection in humans most especially in the tropics where malaria is endemic.

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