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Full length Research Article

Antagonistic Effect of Vitamin E on the Efficacy of Artesunate against *Plasmodium berghei* Infection in Mice

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ABSTRACT

The effect of antioxidant (Vitamin E) on the efficacy of Artesunate was investigated using Plasmodium berghei infected mice. Fifty (50) adult albino mice weighing between 15-25g were used for this study. There were five groups of ten animals each per group. Group I was the normal control group without the parasite and untreated, group II was infected with malaria parasite and 0.9% normal saline was administered, group III was infected with the parasite and treated with artesunate, group IV was infected with the parasaite and vitamin E was administered and group V was infected with the parasite and combination of artesunate and vitamin E were administered. Parasitaemia level and haematocrit (PCV) were monitored upon the administration of antimalaria drug - artesunate, vitamin E, normal saline and combination therapy of both vitamin E and artesunate. The life span of the infected mice was found to be between the 7^{th} and the 10^{th} day post inoculation, while the LD_{50} of the parasite was 156626.2 parasite/µl of blood. Artesunate was observed to rapidly clear the parasite with parasitaemia level of 21632.4 ± 513.3 parasite/µl on the 6^{th} day, 11209.9 ± 363.7 parasite/µl on the 7^{th} day, 1359.7 ± 14.3 parasite/µl on the 8^{th} day, 7.8 ± 2.4 parasite/µl on the 9^{th} day and zero parasitaemia on the 10^{th} day. The effect of artesunate was significantly reduced when co-administered with vitamin E ($p < 0.05$) with parasitaemia 30542 ± 362.5 parasite/µl on the 6^{th} day, 24705.2 ± 489.9 parasite/µl on the 7^{th} day, 15485.0 ± 563.2 parasite/µl on the 8^{th} day, 947.6± 37.8 parasite/µl on the 9^{th} day and 8.0 ± 2.7 parasite/µl on the 10^{th} day. The study suggests that co-administration of vitamin E with artesunate could reduce the efficacy of artesunate in malaria infection.
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Keywords: *Plasmodium berghei, Parasitaemia, Artesunate, Vitamin E, mice.*

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INTRODUCTION

Malaria represents a medical emergency because it may rapidly progress to complication and death without prompt and appropriate treatment (Trampuz et al, 2003). Malaria infection remains a devastating global problem; with an estimated 300-500 million cases occurring annually and 700,000-2.7 million people die of malaria each year (United States Malaria Surveillance, 2000).

Worldwide, the control of malaria has witnessed a serious deterioration (Fernex, 1985). The widespread emergency of parasite strains resistant to the usual antimalarial drug, development of insecticide resistance in mosquito, and reduced insecticide spraying because of economic consideration, are some of the reasons for this deterioration in malaria control. The expert committee on malaria of the World Health Organisation (WHO, 1985) observed that this deterioration would likely continue until novel approaches to the malaria problem are developed.

Severe malaria is almost exclusively caused by *Plamodium falciparum* in humans, but other forms of Plasmodium include *P. vivax, P. ovale* and *P. malariae.* However, different species of the Plasmodium have also been identified in other animals (Jervis et al, 1968). The parasite capable of infecting mice is *P. berghei* (Anigbogu et al, 1997) and produces a disease thought to be a close replica of malaria infection in man (Dosowitz et al, 1976; Franz et al, 1987).

Both nutritional and pharmacological treatments have been utilized to exploit the oxidative stress imposed on the host red blood cell (RBC) by the parasite (Lavender et al, 1989). One of the currently recommended antimalaria drug is Artemisinin (Trampuz et al, 2003), which is derived from Chinese traditional medicine and represents a totally new class of promising antimalaria agents (Klayman et al, 1985) that are active against chloroquine resistant *P. falciparum* (Chawira et al, 1986).

This compound bears an endoperoxide grouping and it acts against the parasite by generating free oxygen radicals in-vivo (Clark et al, 1983).

Vitamin E is an antioxidant which is a nutritional supplement derived from dietary sources (Shohani et al, 1997). They are special group of macronutrrients that protect the body from a destructive process and promote good health by slowing down the ageing process and delaying the onset of many chronic diseases. They act via removal of oxygen and scavenging reactive oxygen species or their precursor (Yossi et al, 2002). Vitamin E has almost become a routine supplement based on its source and health benefits.

Thus, statistics have shown the prevalence of dietary supplement consumption including vitamin E to be between 36 to 51% of adult (Slesinski et al, 1995) and 43% of children between 2-6 years (Moss et al, 1989). The integral proportion of vitamin E out of the dietary suuplement consumed has been reported to be up to 6% (Ervin et al, 1995).

However, the potency of artemisin in the treatment of malaria infection may be affected when co-administered with other nutritional supplement especially vitamin E. The aim of the study is to evaluate the effect of co-administration of vitamin E and artesunate in mice infected with *P. berghei.*

MATERIALS AND METHODS

Adult albino mice weighing between 15-25g were housed in groups of ten animals per group in cages constructed of stainless steel and plastic. They were kept under hygienic condition in the Pharmacology laboratory of the College of Medicine, University of Lagos where the experiment was carried out. The animals were fed on high quality mice diet, water ad libitum.

The parasite was obtained from Nigerian Institute of Medical Research, Yaba, Lagos and the drugs used (Artesunate and Vitamin E) were obtained from NIMET Pharm. Ltd, Ojuelegba, Lagos. The animals were infected with *P. berghei* through intraperitoneal route. The life span of the infected mice without treatment was predetermined using two groups of animals with ten mice in each

group. The group II was infected with the parasite and the course of death of the animals was monitored, while the other group served as the control group. The LD_{50} of parasite in the infected mice was predetermined using probit analysis method. The experimental procedure for the determination of parasitaemia level and packed cell volume involved five groups of ten animals each (group I - V).

The group I animals were administered 0.9% normal saline. Parasitaemia level and packed cell volume of the animals were monitored without infecting the animals with the malaria parasite, *P. berghei*.

The group II animals were infected with *P. berghei* and 0.9% of normal saline was administered to the animals orally starting on the fifth day post infection. The parasitaemia level and packed cell volume of the animals were monitored. The group
III animals were infected with *P. berghei*, and therapeutic dose of Artesunate (5 .0mg/kg on day one and 2.5mg/kg for the next four days) was given to the animals orally starting on the fifth day post infection. The parasitaemia level and packed cell volume of the animals were monitored. The group
IV animals were infected with *P. berghei*, and therapeutic dose of vitamin E (100mg/kg/day over a period of 5 days) was administered to the animals orally starting on the fifth day post infection. The parasitaemia level and packed cell volume were monitored. The group V animals were infected with^{P}. *berghei*, and therapeutic doses of Artesunate and Vitamin E were administered orally starting on the fifth day post infection. The parasitaemia level and packed cell volume of the animals were monitored.

The animals were infected with parasites by obtaining parasitized blood from the cut tip of the tail of an infected mouse. About 0.1ml of infected blood (3 - 4 drops) was diluted in 0.9ml of sterile saline (0.9% NaCl). The mice were inoculated intraperitoneally with 0.1ml parasitized saline suspension.

Development of parasitaemia was monitored by microscopic examination following the method of Fern, McNurtan and Garlick (Shida et al, 1989).

Infected red blood cells were counted using the formula (WHO, 1985):

$$\frac{\text{No of Parasite Counted x 8,000}}{\text{200 WBC}}$$

Haematocrit (PCV) was determined using the heparinized capillary tubes, Hawksley microhaematocrit centrifuge and the microhaematocrit reader (Hawksley and Sons Ltd, Sussex, England).

Statistical analysis: Results obtained from this investigation were subjected to statistical analysis using the student's t-test to test for significance at 0.05 probability level. The results show mean ± Standard deviation of the data.

RESULTS

Parasitaemia was noticeable as early as third day post inoculation. Fig 1 shows that the level of parasitaemia increased with days, with the lowest level 260 parasites per microlitre of blood recorded on the third day while the highest level 165200/µl of blood recorded on the tenth day. However, on the seventh day when the level of parasitaemia increased to 90400 parasites per microlitre of blood, the animals started to die with the highest death recorded on the eighth day with parasitaemia of 130100 parasite per microlitre of blood. Figure 2 showed the LD_{50} of the parasite in the infected mice to be 156626 parasites per microlitre of blood

Table 1 shows the level of parasitaemia of *P. berghei* infected mice administered with different drugs. There was no inoculation of the parasite into the mice in group I, and no parasite was found in their blood. However, there was a progressive increase in parasitaemia in group II animals which served as the parasite control that was infected with the parasite, but treated with normal saline.

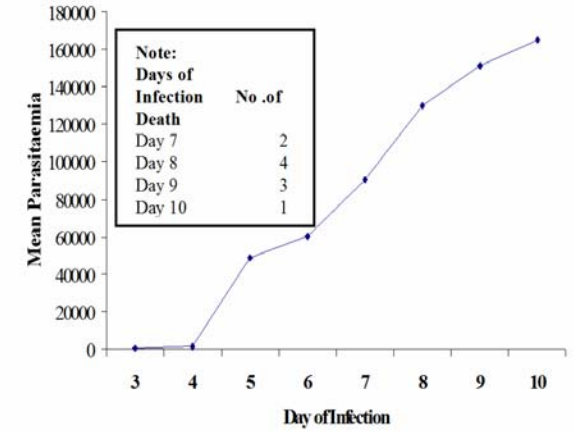

Fig. 1.
Profile of parasitaemia in untreated mice

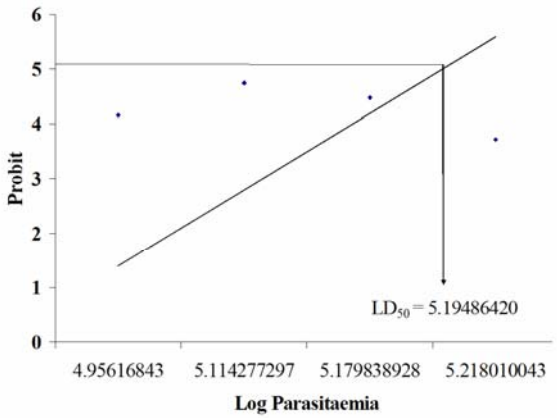

Fig. 2
Probit analysis for the Determination of LD_{50} of Parasite in the infected mice

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Table 1:
Pattern of the Mean Parasitaemia of P. *Berghei* Infected Mice Administered with Different Drugs

Group	Drug used	Mean Parasitaemia Per Day after Inoculation with *P. Berghei*							
		3	4	5	6	*7*	8	*9*	10
I(Normal control)	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
II (Parasite control)	0.9% Normal Saline + Parasite	252.5 ± 6.8	1492.5 ± 35.7	48096[1D] ± 670.5	58781.11 ± 1245.2	83673.75[1D] ± 469.6	110135[4D] ± 5.8	176246.3[4D] ± 379.3	
III	Artesunate + Parasite	230.5 ±8.5 $P_{II/III}$ <0.05	1548 ±8.8 PII/III <0.05	45656.5 ± 381.3 PII/III < 0.05	21632.4 ± 513.3 PII/III < 0.05	11209.9 ± 363.7 0.05	1359.7 ± 14.3 PII/III < 0.05	7.8 ± 2.4 PII/III < 0.05	0
IV	Vitamin E + Parasite	278 ± 12.5 $P_{I/IV}$ < 0.05	1574.4 ± 20.9 $P_{I/IV}$ < 0.05	51841.88[2D] ± 338.9 $P_{I/IV}$ < 0.05	58919[4D] ± 334.5 $P_{I/IV}$ > 0.05	80900[4D] ± 247.5 $P_{I/IV}$ < 0.05			
V	Artesunate + Vitamin E + Parasite	260 ± 12.9 PII/V > 0.05 PIII/V > 0.05	1476 ± 51.3 PII/V > 0.05 PIII/V < 0.05	55910.4 ± 124.8 PII/V < 0.05 PIII/V < 0.05	30542 ± 362.5 PII/V < 0.05 PIII/V < 0.05	24705.2 ± 489.9 $PI_{I/}V$ < 0.05 PIII/V < 0.05	15485 ± 563.2 PII/V < 0.05 PIII/V < 0.05	947.6 ± 37.8 P_{nA}, < 0.05 PIII/V < 0.05	8 ± 2.7 PIII/V < 0.05

Parasitaemia on days 1 and 2 = 0; Parasitaemia per day = Mean ± S.D; D = Number of death; Pyn = P value between group II and III using student T test; PI_{I}/IV = P value between group II and IV using student T test; PI_{I}/V = P value between group II and V using student T test; PIII/V = P value between group III and V using T-test

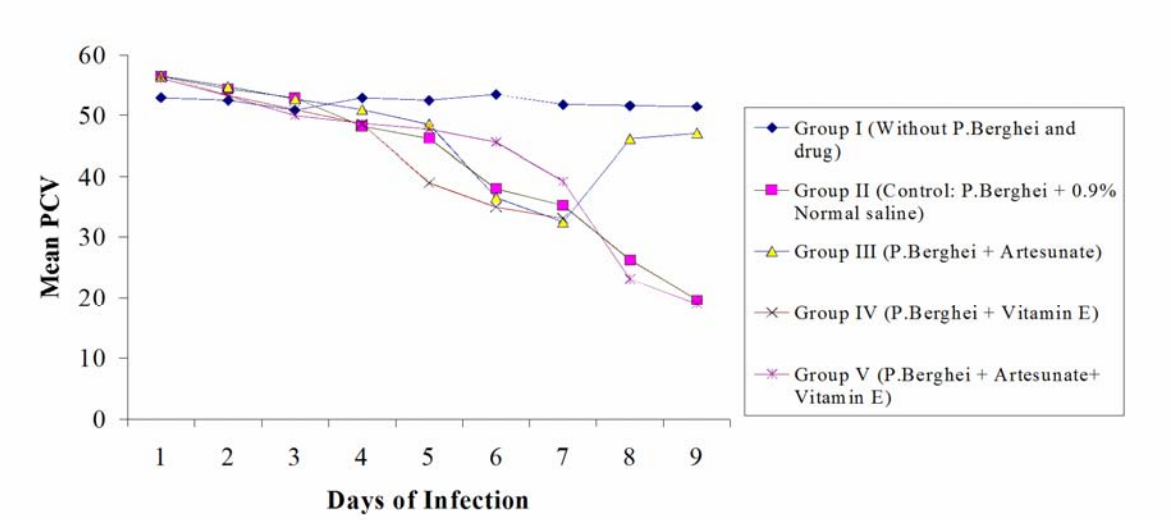

Fig. 3
Haematocrit of *P. Berghei* infected mice administered with different drugs

The results showed mean parasitaemia 252.5 + 6.8/µl on the third day, 48096.0 + 670.5/µl on the fifth day when the animals started to die and 176246.3 + 379.3/µl on the ninth day with the highest number of death. Group III shows a progressive increase in parasitaemia from the third day of inoculation with parasitaemia 230.5 + 8.5/µl to the fifth day with parasitaemia 45656.5 + **381.3/µl** and a gradual decrease after commencing treatment with Artesunate on the 5th day to parasitaemia 216324.0 ± 513.3/µl on the sixth day, 7.8 + 2.4/µl on the ninth day and finally zero parasitaemia on the 10th day

There was a progressive parasitaemia in group IV with value increasing from 278.0 + 12.5/µl on the third day to 51841.98 ± 339.0/µl on the fifth day when the animals started to die even when vitamin E was administered and to a peak parasitamia 80900.0 + 247.5/µl on the seventh day when all the animals died. Group V animals were treated with both artesunate and vitamin E and the result shows a progressive increase in parasitaemia from the third day with parasitaemia 260.0 ± 12.9/µl to 55910.4 ± 124.8/µl on the fifth day after which a gradual decrease in parasitaemia on the sixth day with parasitaemia 30542.0 + 362.5/µl to 947.6 + 37.8/µl on the ninth day and finally 8.0 + 2.7/µl on the 10th day was observed. Table I further shows that the decrease in parasitaemia in group V was not as remarkable as in group III. Thus, there was a significant difference ($P < 0.05$) between the effects of these drugs on the animals from day 5 to day 10. Moreso, group II and III showed a statistical difference ($P < 0.05$) from day 3 to day 9.

As shown on fig. 3, the PCV of the animals in group I shows a reduction in values from the 1st day of the experiment to the third day and increased slightly between the 4th and 6th day and then slightly decreased afterwards to the last day. However, in group II animals, an obvious decrease in value from 56.5 + 0.6% on the first day to 19.6 + 1.6% on the ninth day was observed. The PCV values of group III animals decreased gradually from 56.5 + 0.8% on the first day to 32.5 + 2.9% on the seventh day and began to rise slightly from 46.1 + 4.6% on the eight day to the ninth day. However, a definite pattern was noticed in group IV treated with vitamin E with maximum value of PCV 56.6 + 1.4% recorded on the first day and thereafter decreased gradually reaching a minimum value of 33.1 + 8.7% on the seventh day. The animals

in group IV showed a similar pattern to those in group II, with PCV values decreasing from the maximum on the 1st day to minimum on the 9th day of experiment. The results further shows that the animals in group III exhibited a significant difference ($P < 0.05$) PCV values on day 4, 5, 8 and 9 from those in group II. Groups II and IV animals also showed a significant difference ($P < 0.05$) in PCV values only on day 5.

Group V shows a progressive decrease in PCV from the first day with 56.1 + 1.2% to the ninth day with 18.9 + 2.2%. However, groups II and V were significantly different ($P < 0.05$) on the 2nd, 3rd, 6th, 7th and 8th day. Group III and V showed significantly different values ($P < 0.05$) on days 3, 6, 7, 8 and 9.

DISCUSSION

The results from this study demonstrate the complex interplay that exists between the malaria parasite, artesunate and antioxidant (vitamin E).

The survival period (life span) of the mice infected with *P. berghei* was determined to be between the 7th and 10th day post inoculation and is similar to the earlier observation of Anigbogu and Fagbure, 1997. However, LD_{50} of the parasite per microlitre of blood is 156626.1/µl, which is in agreement with the mortality pattern in group II of animals infected with the parasite and treated with 0.9% normal saline. The death pattern of group IV animals that were administered with vitamin E showed a faster mortality rate over days. This could be suggestive that the antioxidant effect of vitamin E on malaria oxidative stress may not be appreciable during the course of infection, thus a prophylactic administration of antioxidant may protect the animals against the oxidative stress that occurs during malaria infection.

The development of parasitaemia observed in the mice used in this study is similar to earlier reports by previous researchers (Franz et al, 1987; Zuckerman and Yoeli, 1954). The parasitaemia was noticeable as early as the third day post inoculation and developed progressively in the parasite control group II. Thus, the death of animals recorded in this group could be due to oxidative stress that the parasite caused on the animals (Postma et al, 1996) and the decreased level of the packed cell volume which was as a result of destruction of red blood cells resulting in anaemia condition (Browne et al, 2001; Zuckerman et al, 1954).

The pattern of parasitaemia in artesunate treated mice showed a rapid clearance of the parasite to zero parasiatemia, which is in accordance with the work of Trampuz et al, 2003 that showed artemisin derivatives as very effective antimalarial drug. Artesunate support the host defense against the parasite (Postma et al, 1996) and acts against the parasite by generating free oxygen radicals in vivo (Lavender et al, 1989).

The group IV animals treated with vitamin E showed an increasing rate of parasitaemia, a decreasing packed cell volume and rapid death of the animals. This observation could be conclusive that vitamin E is not an antimalarial drug. However, Krungrai and Yuthavong, 1987 showed vitamin E to be an antioxidant and acts against oxygen tension of the host. Thus, a prophylactic treatment with antioxidant (vitamin E) to prevent generation of reactive oxygen species may prevent vascular pathology to the animals and exhibit anti-parasitic activity (Postma et al, 1996).

The result obtained in group V demonstrate vitamin E to antagonised the antimalarial effect of artesunate. This result is consistent with the hypothesis that artesunate (pro-oxidant) acts against the parasite by generating free oxygen radicals and antioxidants (Vitamin E) potentially counteract the effects of pro-oxidants (Postma et al, 1996). This antagonism might be due to the pattern of action of these agents on the host. Thus, the effect of artesunate on malaria parasitaemia in group III is more effective than the co-administration of artesunate and vitamin E in group V ($P < 0.05$).

In conclusion, the study has demonstrated that vitamin E can reduce the efficacy of artesunate in malaria therapy. Thus, co-administration of artesunate and nutritional supplements that contain vitamin E may have a

far reaching consequences on efforts in controlling malaria infection in Africa. Therefore, clinical efforts should be made to control the concurrent use of artesunate and vitamin E as this may affect the treatment goal in malaria therapy.

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REFERENCES

Anigbogu C.N and Fagbure O. A. (1997): *Plasmodium berghei* malaria infection produced more changes in the blood and organ of the mice than rats. *Nigeria Quarterly Journal of Hospital Medicine. 7(1):* 85-88,.

Browne E.N, Mande G.H., Bimka F.N (**2001)**.: The impact of insecticide treated bednets on malaria and anaemia in pregnancy. *Tropical Medicine and International Health. 6(9):* 667-676,.

Chawira A.N., Warhurst D.C, Peters W. (1986): Quinghaosu resistance in rodent malaria. *Trans R Soc Trop Med Hyg.*80:477-80,.

Clark I. A, Cowden W.B and Butcher G.A(1983).: Free oxygen radical generators as antimalarial drugs. *Lancet. 1:234,*

Dosowitz R.S and Barnwell J.W (1976): *Plasmodium berghei.* Deep vascular sequestration of young forms in the heart and kidney of the white rat. *Ann. Trop. Med. Parasitol* 70:475-476,.

Ervin R.B., Wright J.D., Kennedy J (1999): Use of dietary supplements in the United States, 1988-1994. National Centre for Health Statistics. Vital Health statistics II (244):1-14..

Fernex M (1985): Mefloquine and its allies. *World Health.* May 6-7,.

Franz D. R., Lee M., Seng L. T (1987):*:* Peripheral vascular pathophysiology of *Plasmodium berghei* infection. A comparative study in the cheek and the brain of golden hamster. *Am. J. Trop. Med Hyg. 36:* 474-480,.

Jervis H.R., MacCullum D.K. and **Sprinz H (1968):** Experimental *Plasmodium berghei* infection in the hamster. **Arch Path.** 86:328-337,.

Klayman D.L: Quinghaosu (Artemisin): An antimalarial drug from China. *Science. 228:* 1049-55, 1985.

Krungkrai S.R, Yuthavong Y (1987): The antimalarial action on Plasmodium falciparum of Quighaosu and Artesunate in combination with agents which modulate oxidant stress. *Trans R Soc trop med Hyg.* 81:710-714,

Lavender O.A., Ager A.L, Morris V.C (1989): Quighaosu dietary vitamin E, selenium and cod liver oil. Effect on susceptibility of mice to the malarial parasite Plasmodiun yoelli. *America Journal of Clinical Nutrition. 5:* 346-3 52

Moss A.J., Levery A.S., Kim I., **Park Y.K.** Use of vitamin and mineral supplements in the United States: Current users, types of products and nutrients. Advance data from vital and health statistics, No. 74. Hyattsville, maryland: national Centre for Health Statistics. 1989.

Shida. K.K, Lewchalermvongse B and Pang L. W: *Plasmodium berghei* malaria infection in causes increased cardiac output in rats, Rattus rattus. **Exp. Parasitol** 68:253-259, 1989.

Shohami E, Yannai E.B, Horowit M and Hohen R. Oxidative stress in closed head injury: brain antioxidant capacity as an indicator of functional outcome. *J. CerebBlood Flow Metab.* 1997, 77:169-184.

Slensinki M.J., Subar A.F., Kahle L.L. Trends in use of vitamin and mineral supplements in the United States. The b1987 and 1992 National Health Interview Survey. J. Am Diet Assoc. 95:921-3. 1995.

Trampuz A, Matjaz J, Igormuzloric Rajesh Prabhu: Severe malaria. **Clinical review. 7(4):** 315-323, 2003.

United States Malaria Surveillance, 2000. WHO composite work plan on malaria. 1985; htt://mosquito.who.int/does/3gpms.comp.litm.

Yossi GS., Ziv R, Eldad M, and Daniel O (2002): Antioxidant therapy in acute central nervous system Injury: *Current State Pharmacol Rev.*, 54:271-284.

Zuckerman and Yoeli: Age and sex as factors influencing *Plasmodium berghei* infections in intact and splenectomized rats J. **Inf. Dis.** 225-236, 1954.