วารสารเทคนิคการแพทย์ เชียงใหม่
BULLETIN OF CHIANG MAI
ASSOCIATED MEDICAL SCIENCES

Vol. 7 No. 1
January 1974

A STUDY OF THE SIZE, GROWTH RATE AND SEROLOGY OF TRYPANOSOMA LEWISI INFECTION IN WHITE RATS *

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ABSTRACT :

The size and growth rate of the organism and serology of T. lewisi infections in white rats was studied. The size as reported by Taliaffero (1921) is within the range found in the present work (30 U in length and 1.5 U in breadth). Parasitaemia appears at around 20 days. In the early stage of growth the organism is polymorphic but later monomorphism prevails. The micro - agglutination test was performed using immune sera from convalescent rats against an antigen of living organisms. Clumping due to adhearance of the posterior ends of the organisms was frequently observed.

INTRODUCTION :

The flagellate, Trypanosoma lewisi, is a non-pathogenic blood parasite occuring in various species of rats all over the world. It is transmitted from rat to rat by fleas. T. lewisi is classified in subgenus of Herpetosoma having an elongate curved body which is moderate in size, tapering at both ends. The large ovoid nucleus is located just anterior to mid body. The small distinct spherical kinetoplast subterminal. The undulating membrane is only slightly convoluted.

In present study, T. lewisi was kindly provided by Prof. Robert G. Yeager of the Department of Parasitology, School of Public Health and Tropical Medicine,

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* Part of this work had been submitted to The Faculty of Medicine, Chiang Mai University in partial fulfillment of the requirement for the B.Sc. (Med. Tech.) degree.

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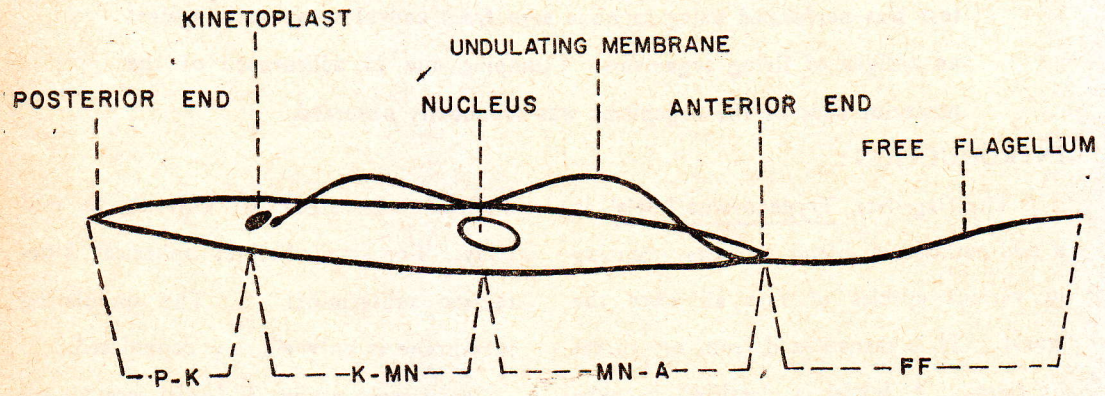
Tulane University. This strain of T. lewisi has been regulary transferred in white rats every 15 days for three years in this laboratory. The present work concerns studies on the size and growth rate of the organisms and serological reactions of the host to infection.

MATERIALS AND METHODS :

Thin blood films were prepared from tail vein blood of white rats infected with T. lewisi in the later phase around 10 days post infection. The blood films were fixed in absolute methanol for 1 minute and stained with dilute Giemsa's stain (1:50) for 45 minutes. The following morphological land marks of typical adult form (Fig. I) were measured : size of kinetoplast and nucleus; the distance from posterior end to the center of kinetoplast (P-K); the distance from center of the nucleus (K-MN); the distance from the center of the nuclus to the anterior end (MN-A); the length of free flagellum (FF); the total length (TL) and the maximum width of parasites (W).

FIGURE I.

DIAGRAM OF Trypanosoma lewisi SHOWING THE PART OF THE ORGANISM AND THE DISTANCES MEASURED IN THIS STUDY



The study of growth rate was performed by both direct and indirect methods.

In the direct method counts of organism were made in known volumes of blood using Toison's fluid * as a diluent.

* Toison's fluid :- Na_2SO_4 ; 8 gm, glycerine 30 ml, crystal violet 10 mg. and distilled water g. s. added to 160 ml.

Peripheral blood from the tail vein was drawn to the 0.5 mark of a calibrated white count pipette, excess blood was wiped off the pipette tip and sufficient Toison's fluid was drawn to bring the total volume up to the 11 mark. The solution was mixed by vigorous shaking and a portion was added to a hemocytometer for enumeration. By touching a drop of solution at the junction of the chamber and cover slip, the solution flowed smoothly into the chamber by capillary attraction. The completely filled chamber was allowed to stand for 2-3 minutes allowing the parasites to settle. For enumerating the total number of parasites, the numbers of parasites in 40 of the smallest squares (for red blood cells counting) was mulipled times 20,000 to the approximate number of parasites per ml. of blood.

Using indirect method, the number of parasites per 100 white blood cells in Giemsa's stained thin blood of tail blood was determined by microscopic examination under the oil immersion objective. At the same time total white blood counts of peripheral blood from the same rat were performed. From these two data the total number of parasites per ml. could be estimated.

The micro-agglutination test was performed. Using two to three ml. of heparinized heart blood collected from an infected rat during the period of peak parasitaemia about 8 days post infection. Phytohaemagglutinin was added to the blood in a final concentration 1%. The blood was centrifuge at 2,000 r.p.m. for 10 minutes. The supernatant plasma was siphoned off and the whitish layer above the sediment was collected. This layer was composed mostly of parasites and some white blood cells. The parasites were washed with normal saline two or three times and resuspended to give a final of approximately 2×10^6 organisms/ml. This suspension served as living antigent.

Immune sera were collected from convalescent rats about 1 month post infection. Prior to the test sera were incubated at 56°C. for 45 minutes to destroy the complement. Serum dilution of 1:5, 1:10 and 1:50 were prepared using normal saline as an diluent. The microagglutination test was performed in "microconcavity slides". Brieft serial dilution of 1:5, 1:10 and 1:50 were prepared and 0.1 ml. of each dilution was mixed with 0.1 ml. of antigen which in consecutive wells. The slide was left at room temperature for 1 hour, after the agglutination reaction was observed under the microscope. The following arbitary criteria were recorded for each dilution.

Degree of agglutination	Description	Floccule size (U)
0	No agglutination	–
1^+	1–5 floccules/field	50–100
2^+	6–10 floccules/field	50–150
3^+	10 floccules/field	100–250
4^+	complete agglutination	250–500

RESULTS.

The measurement of various structures of 115 parasites are given in Table I.

Table I

Various morphological parameters (U) of 115 Trypanosoma lewisi organisms, as measured with the occular micrometer.

Description	Range	Mean	Standard deviation	Coefficient of variation
KINETOPLAST				
width	0.3–1	0.617	0.59	1.91
length	0.4–1.5	0.966	0.84	0.833
NUCLEUS				
width	1.3	1.452	1.79	1.232
length	2.5–4	2.665	1.880	0.78
P–K	2–11	4.67	3.98	1.653
K–MN	5–4	9.65	4.042	0.481
MN–A	3.13	7.513	4.18	0.556
FF	5- 13	8.078	4.939	0.611
TL	17–40	29.521	8.86	0.30
W	1.5–8	3.026	3.544	1.171

The growth rates determined by direct and indirect methods are illustrated in Fig. II. The parasites were first detected in the blood on the second day and reached a peak of parasitaemia on the eighth day of infection. Thereafter parasitaemia gradually decreased and disappeared on twentieth day of infection.

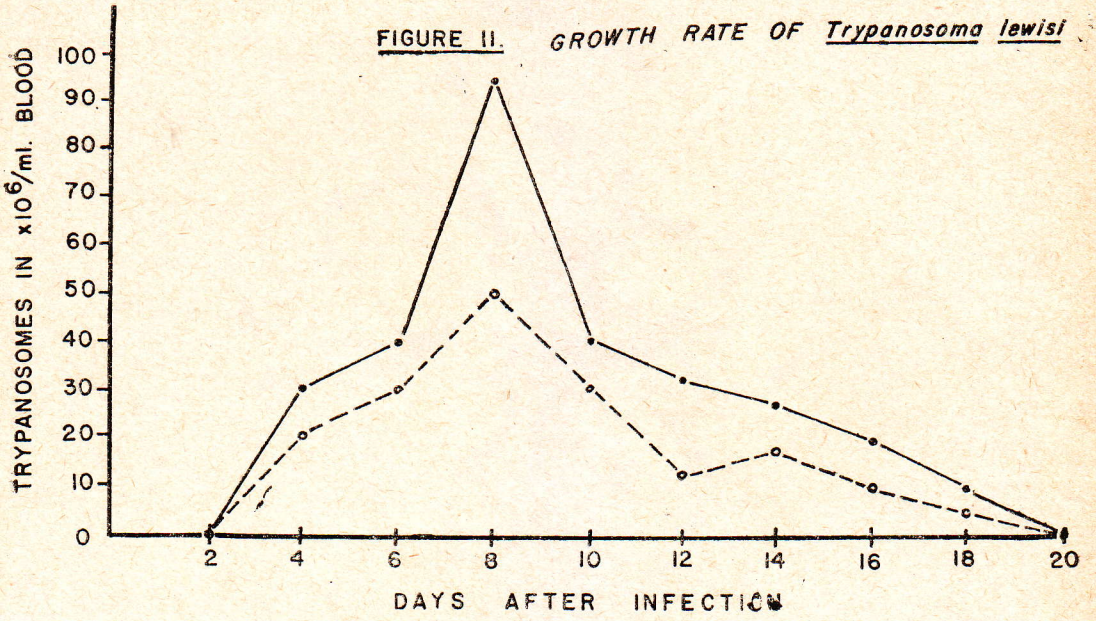


FIGURE II. GROWTH RATE OF *Trypanosoma lewisi*

The results of the study on micro-agglutination are given in Table II. The form of the characteristic agglutination clumps are shown in Fig. III.

Table II. The result of micro-agglutination. Determination using sera from convalescent rats previously infected with **T. lewisi**

No. of rate	Control	Degree of agglutination		
		1:5	1:10	1:50
9	0	4^+	4^+	0
10	0	4^+	4^+	4^+

FIGURE III. *TYPICAL AGGLUTINATION CLUMP IN IMMUNE SERUM*

DISCUSSION :

The results of measurement of various structure of T. lewisi in the present work vary slightly from those reported in 1921 by Taliaferro (P-K 4.268 ± 0.36 ; K-MN. 10.854 ± 0.016 ; MN-A 9.511 ± 0.047 ; FF. 6.619 ± 0.068 ; TL 31.25 ± 0.059 W 1.590 ± 0.015). Variation in size may be due to the technique of measurement. In the present study measurements were made with a calibrated ocular microscope. It appears that there is some variation of measurement in this method as compare to use of the camera lucida or by direct photography.

Studies of growth rate both direct and indirect methods demonstrated the appearance of organism in the blood on the second day of infection, followed by a peak parasitaemia on the day eight. Parasites were not recovered in infected rats after 20 days post infection. The authors were not able to prolong the duration of parasitaemia in rats up to 36 days as was reported by Taliaferro (1921).

Observation while making micromeasurements of various structures from thin blood films revealed the that up to eighth day of infection the parasites showed a marked polymorphism. It is believed that the examination of the growth curves (Fig. II) indicated that the period of polymorphism coincides with period of exponential growth and multiplication

After eight days of infection, it appears that multiplication of parasites is completed and parasitaemia begin to gradually decrease. During this period and continuing for about 12 days the organism appears to be monomorphic.

In the study of micro-agglutination phenomenon, it was observed that during the first 10 minutes of the reaction of the organisms moved vigorously in the antisera. Thereafter the organisms began to adhere forming clumps which reach a maximum size of 250 U within one hour. The clumping or agglutination phenomenon was observed to involve a characteristic series of events. Within the first ten minutes the affected organisms appeared to become sticky and began to adhere to one another. Thereafter posterior portion of the body began to adhere, and leaving the anterior half of the body free. In this manner clumps of organisms look on various configurations, occasionally resembling posterior rosette-like forms to confirm the clumping in posterior rosettes mentioned by Chandler (1961).

ACKNOWLEDGMENT :

The authors are grateful to Dr. Chirasak Kamboonruang and Mr. Micheal Klicks for their helpful discussion throughout this report.

REFERENCES :

1. Belding, David Le, 1965. Text book of Parasitology : 146-147.
2. Chandler, Asa. C., and Clark P. R. 1962. Text book of parasitology : 133-137.
3. Chandler Asa. C. 1958 "Some considerations relative to the nature of immunity in **Trypanosoma lewisi** infections, J. Parasitology : 129-135.
4. Molyneux, David H. 1969. Intracellular stages of **Trypanosoma lewisi** in fleas and attempts to fine such stages in other **Trypanosoma** species, Parasitology, 59 : 737-744.
5. Eyles, Don E. 1952. Incidence of **Trypanosoma lewisi** and **Hepatozoon muris** in the Norway rats, J. Parasitology, 38 : 222-225.
6. Fong, Yap Loy, Sandosham, A.A. and Loong, Seow Clin. 1967, The polymorphism of **Trypanosoma lewisi**. The medical Journal of Malaya, 4 : 369.

7. Hilton,, D.F.J. and Mahrt, J.L 1972. Taxonomy of **Trypanosomes** (Protozoa: Trypanosomatidae) of spermophilus spp. (Rodentia : Sciuridae Parasitology, 65 : 403-425.

8. Judge, David M. and Anderson M. Sidney. 1964, Ultrastructure of **Trypanosoma lewisi**, J. Parasitology, 50 : 757-762.

9. Kartman, Leo. 1954. Observation on **Trypanosoma lewisi** and **Grabamella** spp. in the blood of rats from Hamaka district, island of Hawaii, J. Parasitology, 40 : 571-588.

10. Lincicome, Davis Richard and Watkins, Roy Cherter 1965. Antigenic relationships among **Trypanosoma lewisi** complex cells, J. Parasitology, 55 : 365-373.

11. Lumsden W.H.R. 1965 Biology aspects of Trypanosomiasis research, Advances in parasitology, 3 : 5-14.

12. Taliaferro, W. H. 1921 Variation and inheritance in size in **Trypauosoma lewisi**" Proc. N.A.S. 7, : 138-143.

13. Soulsby, E J.L. 1968. Textbook of helminths, Arthropods and Protozoa of Domesticated Animals. : 556 557.

14. Swellengrebel N.H. and Sterman M.-M. 1961 Textbook of Animal Parasites in Man. : 58-61.

ย่อเรื่อง

การศึกษาอัตราการขยายพันธุ์ และการ ทดสอบปฏิกริยาทางน้ำเหลืองของ T lewisi ได้ทราบว่ามีขนาดเฉลี่ย กว้าง 1.5 ไมครอน ยาว 30 ไมครอน หนูขาวติดเชื้อนี้ได้นาน 20 วัน แล้วเชื้อนี้ก็จะหมดสิ้นไป การทดสอบปฏิกริยาทางน้ำเหลืองของหนูที่ฟื้นจากการติดเชื้อนี้แล้ว กับตัวเบื่น ๆ ได้ทำให้เชื้อเจับกันเป็นกลุ่มก้อน โดยใช้ส่วนด้านหลังของตัวติดกัน ใน เวลา 1 ชั่วโมง ที่อุณหภูมิห้องจะตายโดยการแตกสลายของตัวเชื้อ