Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. *87* (4): 511-516, oct./dec. 1992

TRYPANOSOMA RANGELI (TEJERA, 1920): OBSERVATIONS UPON PLEOMORPHISM

S. URDANETA-MORALES & F. TEJERO

Seccion de Parasitologia, Instituto de Zoologia Tropical, Facultad de Ciencias,
Universidad Central de Venezuela Apartado 47058, Caracas, Venezuela

Metatrypomastigotes of Trypanosoma rangeli *Tejera, 1920, harvested from LIT medium, were inoculated i.p. or s.c. into 6, 16, and 26 g NMRI mice, these representing increasing degrees of immunological maturity. In all cases, similar pleomorphic patterns were observed. Four morphobiometrically differentiable types of trypanosome were encountered in an overlapping temporal sequence. These observations, taken in comparison with those on pleomorphism in this and other species of* Trypanosoma *by other workers, are consistent with the hypothesis that the pleomorphic types represent the natural development of the parasite, rather than the result of the immune response of the mammal host. Small, slender trypanosomes prevalent at the onset of the parasitemia either reinvade the tissue cells for relatively limited subsequent generations of tissue reproduction, or else differentiate toward the forms that are only capable of colonizing the insect vector.*

Key words: *Trypanosoma rangeli* – pleomorphism – biometrics

Trypanosoma rangeli is a hemoflagellate that employs a wide variety of American mammal hosts and hemipterous insects (Reduviidae, Triatominae) in its life cycle (D'Alessandro, 1976). Its behavior in the insect vectors is well understood (see references in Tovar et al., 1989; Hecker et al., 1990); however, studies on its development in the mammal have given controversial results (see references in Urdaneta-Morales & Tejero, 1986; Scorza et al., 1986).

Morphological studies of bloodstream trypomastigotes of *T. rangeli* have been made mainly in isolated stages during the course of natural infections of humans (De Leon, 1949; Torrealba et al., 1950; Pifano, 1954) and in animals, both domestic (Pifano et al., 1949) and wild (Pifano, 1954; Walton & Sousa, 1967); experimental infections have also been studied (Miles et al., 1983; Steindel et al., 1991). Grewal (1969) and Añez (1981) have reported certain progressive morphological changes in the parasite in experimental infections.

Using our mouse model (Urdaneta-Morales & Tejero, 1985; Tejero et al., 1988) we have studied the influence of host age and route of inoculation upon the course of pleomorphism (Lom, 1979) in *T. rangeli.*

MATERIALS AND METHODS

Three groups of 10 male albino mice (NMRI strain), with mean weights of 6, 16, and 26 g, were inoculated i.p. with 100 000 metatrypomastigotes/g body weight (Tejero et al., 1988) of the "Dog-82" strain of *T. rangeli* harvested from axenic LIT medium (Urdaneta-Morales & Tejero, 1985). An additional group of 10 6 g mice was inoculated s.c.

From each group of 10 mice, tail blood was taken from 5 mice chosen at random every 24 hr for examination fresh (Urdaneta-Morales & Tejero, 1985) and for preparing stained films. Parasitemias were determined in this way (Brener, 1962) until the parasites were no longer detectable in fresh blood. Thin films were fixed for 3 min in absolute methanol and stained for 30 min with Giemsa. The stained films were examined under oil immersion (1000 X) by 2 independent observers; a minimum of 50 randomly chosen flagellates in each stage of parasitemia were drawn with a "Nikon" camera lucida to determine the biometrics

This study was supported by the Consejo de Desarrollo Cientifico y Humanistico de la Universidad Central de Venezuela – Grant No. CO3 31 1926 91.

Received 18 March 1992.
Accepted 10 April 1992.

according to Hoare (1972). By cluster analysis, employing the values of the total length of the flagellates, there were established the four pleomorphic types, examples of which were photographed as already described (Urdaneta-Morales & Tejero, 1985). In periods of low parasitemia, a minimum of 500 fields were examined.

RESULTS

The "Dog-82" strain of *T. rangeli,* in all three groups of mice of differing weights and with both routes of inoculation, showed the typical patterns of parasitemia (Fig. 1) and similar morphological development. As the infections progressed, there appeared an overlapping sequence of four morphologically differentiable types of blood trypanosome (Fig. 2), clearly separable biometrically (Tables I and II). Type 1 had an undulating membrane and vacuoles in the cytoplasm that were scarcely perceptible, while Type 4 had an undulating membrane with 4-5 waves and numerous vacuoles in the cytoplasm; the intermediate Types 2 and 3 showed progressive development of the membrane and of cytoplasmic vacuolization. Types 1-3 were present at the beginning of parasitemia and during its rise; Types 3-4 abounded during the peak and as the parasitemia fell; Type 4 was the only form present prior to the disappearance of parasitemia (Figs 3A, B, C, D).

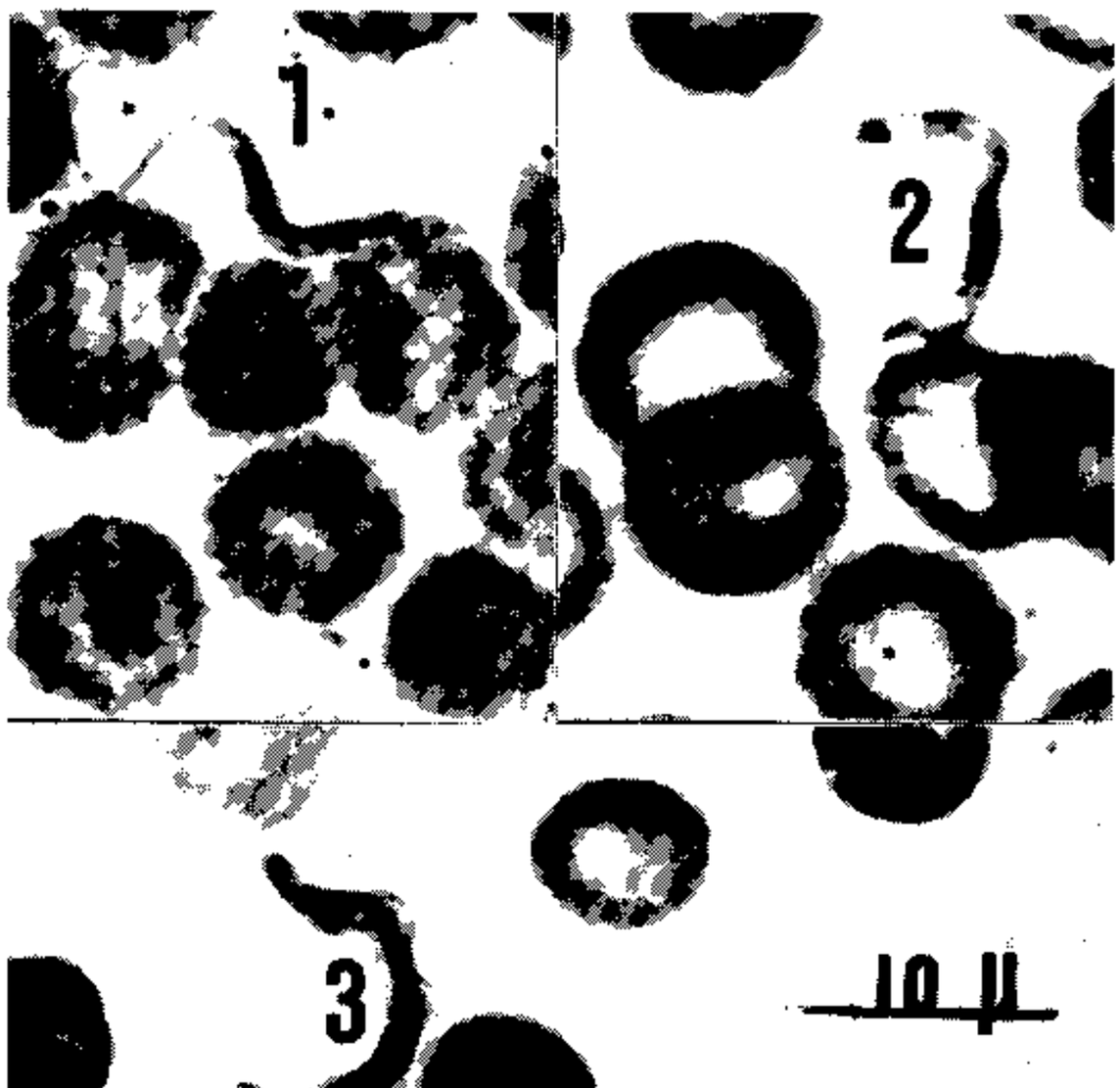

Fig. 2: pleomorphic types (1-4) of *Trypanosoma rangeli* from blood of infected mice. (Giemsa. 1,500 X).

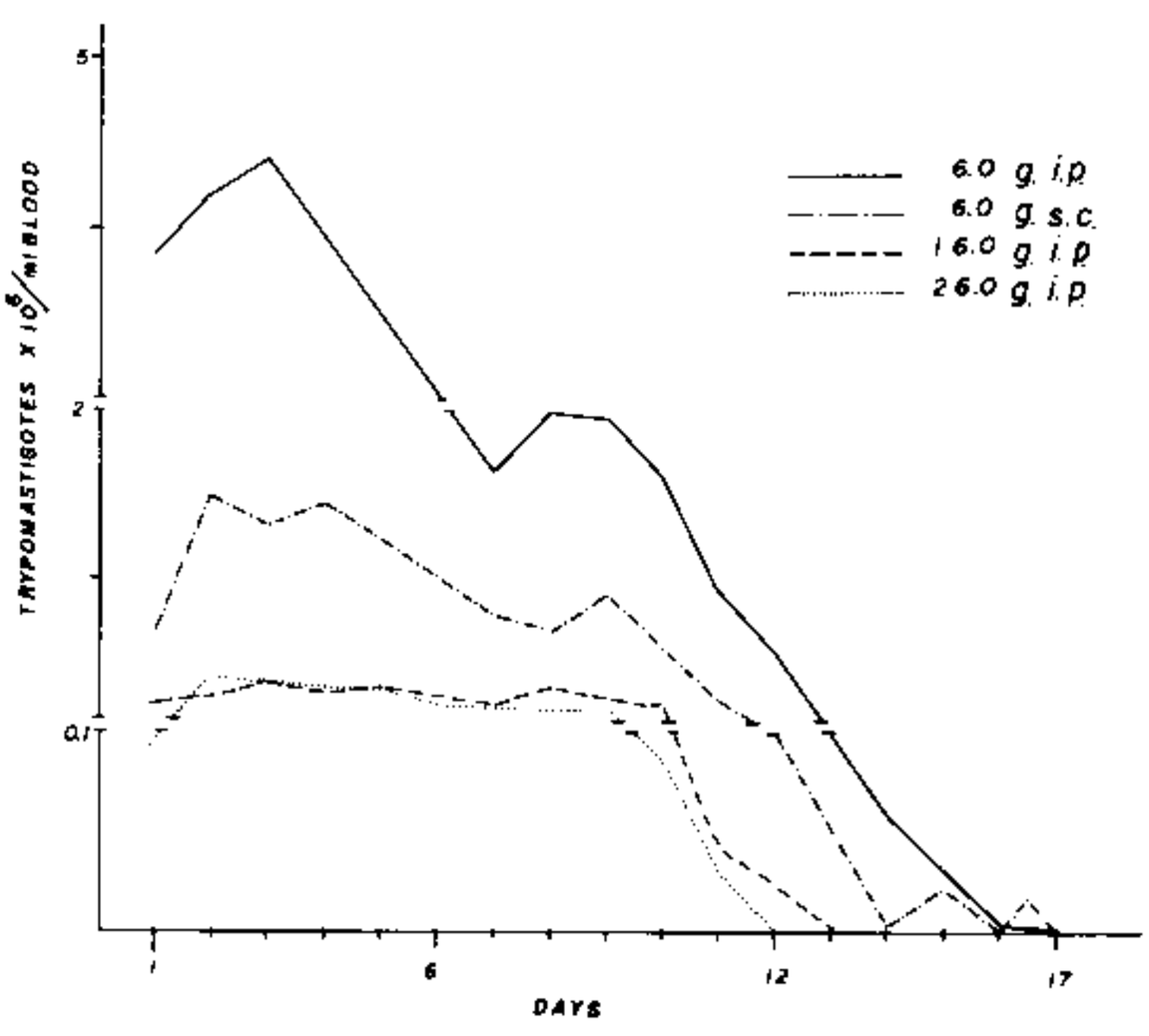

Fig. 1: parasitemias by *Trypanosoma rangeli* in white mice of different weights, inoculated intraperitoneally or subcutaneously. Termination of the curve indicates disappearance of parasites.

DISCUSSION

The phenomenon of cellular variability by which a single genotype may originate different phenotypes is often a consequence of environmental conditions influencing cellular differentiation (Overath et al., 1983). Pleomorphism, a particular expression of variability, is defined by Lom (1979) as "a sequential phenotypic manifestation (at times superimposed) of a genotype expressed in the stage of the trypomastigote in the vertebrate host". This phenomenon, observed in blood flagellates of poikilothermic (Lom, 1979) and homoiothermic vertebrates (Nantulya et al., 1976; Brener, 1979; Ormerod, 1979; Urdaneta-Morales, 1983) is manifested by the successive expression, possibly programmed, of different genes (Vickerman, 1985). Changes in overall size, ratio of length to width, in the number and size of the waves in the undulating membrane, in the positions of the nucleus and the kinetoplast, and in the length of the free flagellum, these are all observable morphological characters expressing the phenomenon (Lom, 1979; Ormerod, 1979).

TABLE I

Biometric comparisons of blood trypomastigotes from infected mice. Ranges given with mean in parentheses

Group No.	Measures (μ)						
	L	B	PK	PN	NA	F	KN
1	9.3 38.9 (32.1)	0.9 5.1 (2.9)	1.1 8.8 (3.7)	5.3 20.7 (15.00)	1.0 12.8 (6.2)	1.6 21.2 (10.9)	3.2 16.7 (11.3)
2	15.0 38.9 (30.7)	0.9 5.5 (2.8)	0.9 7.8 (3.6)	7.6 22.0 (14.8)	0.9 13.0 (6.2)	0.01 22.2 (9.7)	3.5 19.0 (11.2)
3	10.1 39.0 (31.5)	0.9 5.0 (2.9)	1.0 9.4 (3.5)	5.2 20.7 (15.6)	1.7 12.4 (6.1)	1.0 18.5 (10.0)	4.1 15.9 (11.2)
4	9.3 39.0 (30.3)	0.9 5.1 (3.0)	0.5 8.0 (3.4)	5.2 22.1 (15.1)	1.0 12.6 (6.0)	1.3 18.6 (9.2)	3.1 17.4 (11.7)

L = total length; B = maximum width; PK = distance between posterior end and kinetoplast; PN = distance between posterior end and nucleus; NA = distance between anterior end and nucleus; F = length of free flagellum; KN = distance between nucleus and kinetoplast.
Group No.: 1) 6 g. i.p.; 2) 6 g, s.c.; 3) 16 g, i.p.; 4) 26 g, i.p.

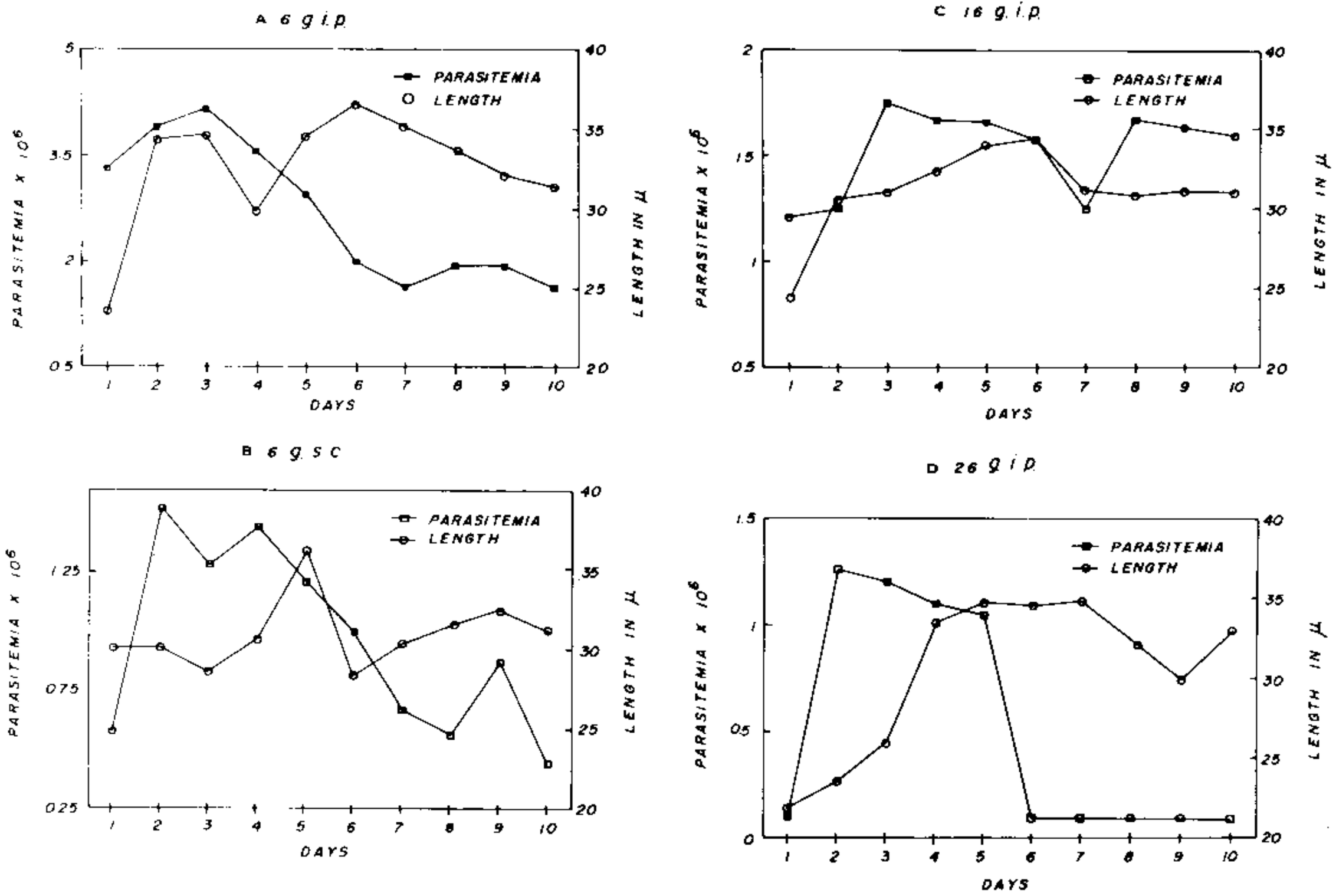

Fig. 3: curves showing comparison between course of parasitemia and mean length of blood trypanosomes in mice: A) 6 g, i.p.; B) 6 g, s.c.; C) 16 g, i.p.; D) 26 g, i.p..

There is much controversy upon the biological significance of pleomorphism in *Trypanosoma* and upon the mechanisms which control it. Some authors have suggested a sexual process, while others take it to be maturation and differentiation in the trypomastigote (see

TABLE II

Biometric data on the total lengths of the pleomorphic types (1 – 4) of *Trypanosoma rangeli* from infected mice

Pleomorphic types	Measures (μ)	
	Range	Mean
1	9.3 – 15.0	10.9
2	16.7 – 21.0	17.9
3	24.1 – 26.9	24.9
4	31.5 – 39.0	31.9

references in Brener, 1979; Urdaneta-Morales, 1983). Others yet have taken pleomorphism to be a result of the aggressive immune response of the mammal host (Pizzi, 1953; Brener, 1969). This last concept, however, appears to be negated by the appearance of pleomorphism in immunologically deficient hosts (Goedbloed & Kinyanjui, 1970; Urdaneta-Morales, 1983), in immuno-suppressed hosts (Balber, 1972; Ormerod et al., 1974; Nantulya et al., 1976; Murray & Black, 1985), and in tissue culture (Brun et al., 1981; Urdaneta-Morales, 1983). In our investigations, we have observed the identical pleomorphic types and their succession in both immunologically immature animals and in adults. This appears to indicate that the pleomorphism is a natural developmental sequence in the bloodstream rather than a consequence of the immune response of the host.

Mitochondrial repression and activation, with the consequent changes in energy metabolism, may govern the morphological changes that occur during the bloodstream cycle of certain species of the Salivarian trypanosomes, with formation of slender and stumpy forms, respectively; structural and/or spatial modification of the microtubules may parallel these physiological changes, thus modifying the architecture of the cytoplasmic membrane (see references in Vickerman, 1985). The pleomorphism in other species (particularly *T. cruzi*) would not necessarily follow this pattern, since there is no pronounced mitochondrial cycle in these forms (Vickerman, 1985), and the ultrastructure of the slender and stout forms of *T. cruzi* is very similar (Maria et al., 1972). The ultrastructural and metabolic significance of these events for the pleomorphism of *T. rangeli* have not yet been elucidated.

Slender forms could possess a greater infectivity for the mammal host (Himuri et al., 1976; Vickerman, 1985), readily penetrating the host cells (Brener, 1979); this would seem to be particularly significant for those species that reproduce in the tissues (subgenera *Schizotrypanum* and *Herpetosoma*). Our results are in accord with these observations; the small, slender Type 1 trypanosomes rapidly disappear from the bloodstream, invading the tissue cells (Urdaneta-Morales & Tejero, 1986), where they reproduce as amastigotes, which transform into trypomastigotes that are liberated into the bloodstream, where they either reenter the tissues for a relatively brief reproductive cycle (Scorza et al., 1986) or else develop pleomorphically. The broad and the stout trypomastigotes of the genus *Trypanosoma* would be preadapted for growth and development in the insect vector (Wijers & Willet, 1960; Brener, 1979; Vickerman, 1985; Ribeiro et al., 1986). In our laboratory, we have found that Type 4 trypomastigotes of the "Dog-82" strain have low infectivity to normal hosts; massive inoculations of this type of trypomastigote into rats and into mice of whatever age produce only transient parasitemias which cannot be sequentially transmitted by injection of parasitized blood into new hosts. These forms however readily infect the nymphs of *Rhodnius prolixus, R. robustus,* and *Triatoma vitticeps,* producing salivary gland infections that are readily transmitted by the insect bite, inducing parasitemias in healthy mice (Urdaneta-Morales & Tejero, 1985; Tovar et al., 1989). These observations raise the question as to whether the cytoplasmic vacuolization seen in the later blood trypanosomes of *T. rangeli* may not be a degenerative process, as postulated for other *Trypanosoma* (Ormerod, 1979), but rather a preadaptation for the takeup by the insect vector, and whether the process of vacuolization might be responsible for loss of virulence to the mammal host.

ACKNOWLEDGEMENT

To Mr Ian McLure for technical assistance and aid in preparing the manuscript.

REFERENCES

AÑEZ, N., 1981. Studies on *Trypanosoma rangeli* Tejera, 1920. I. Deposition, migration and growth of *T. rangeli* in two mammals. p 19-25. In *Parasitological Topics, Spec. Publ. No. 1, Soc. Protozool.,* Allen Press, Kansas.

BALBER, A., 1972. *Trypanosoma brucei:* Fluxes of the morphological variants in intact and X-irradiated mice. *Exp. Parasitol., 31:* 307-319.

BRENER, Z., 1962. Therapeutic activity and criterion of cure on mice experimentally infected with *Trypanosoma cruzi. Rev. Inst. Med. Trop. São Paulo, 4:* 389-396.

BRENER, Z., 1969. The behavior of slender and stout forms of *Trypanosoma cruzi* in the blood-stream of normal and immune mice. *Ann. Trop. Med. Parasitol., 63:* 215-220.

BRENER, Z., 1979. O parasito: relacoes hospedeiro-parasito. p. 1-41. In Z. Brener & Z. Andrade (eds) *Trypanosoma cruzi e Doenca de Chagas,* Guanabara Koogan, Rio de Janeiro, Brasil.

BRUN, R.; JENNI, L.; SCHONENBERGER, M. & SCHELL, K., 1981. *In vitro* cultivation of blood-stream forms of *Trypanosoma brucei, T. rhodesiense,* and *T. gambiense. J. Protozool., 28:* 470-479.

D'ALESSANDRO, A., 1976. Biology of *Trypanosoma (Herpetosoma) rangeli* Tejera, 1920. p. 328-403. In W. H. R. Lumsden & D. A. Evans (eds) *Biology of the Kinetoplastida,* Vol 1, Academic Press, New York.

DE LEON, J. R., 1949. El *Trypanosoma rangeli* observado en seres humanos en Guatemala. *Pub. Inst. Inv. Cient.* No. *3* (Guatemala).

GOEDBLOED, E. & KINYANJUI, H., 1970. Development of African pathogenic trypanosomes in chicken embryos. *Exp. Parasitol., 27:* 464-478.

GREWAL, M. S., 1969. Studies on *Trypanosoma rangeli,* a South American human trypanosome. *Res. Bull. Panjab Univ., 20:* 449-486.

HECKER, H.; SCHWARZENBACH, M.; RUDIN, W., 1990. Development and interactions of *Trypanosoma rangeli* in and with the reduviid bug *Rhodnius prolixus. Parasitol. Res., 76:* 311-318.

HIRUMI, H.; DOYLE, J. J. & HIRUMI, K., 1976. African trypanosomes: Cultivation of animal-infective *Trypanosoma brucei in vitro. Science, 196:* 992-994.

HOARE, C. A., 1972. *Herpetosoma* from man and other mammals: *Trypanosoma (Herpetosoma) rangeli* Tejera, 1920. p. 288-313. In *The trypanosomes of mammals: a zoological monograph.* Blackwell Scientific Publications, Oxford.

LOM, J., 1979. Biology of the trypanosomes and trypanoplasms of fish. p. 269-337. In W. H. R. Lumsden & D. A. Evans (eds) *Biology of the Kinetoplastida,* Vol. 2, Academic Press, New York.

MARIA, T. A.; TAFURI, W. & BRENER, Z., 1972. The fine structure of different bloodstream forms of *Trypanosoma cruzi. Ann. Trop. Med. Parasitol., 66:* 423-431.

MILES, M. A.; ARIAS, J.; VALENTE, S. A.; NAIFF, R. D.; SOUZA, A. A.; POVOA, M. M.; LIMA, J. A. & CEDILLOS, R. A., 1983. Vertebrate hosts and vectors of *Trypanosoma rangeli* in the Amazon basin of Brazil. *Am. J. Trop. Med. Hyg., 32:* 1251-1258.

MURRAY, N. & BLACK, S. J., 1985. African trypanosomiasis in cattle: working with Nature's solution. *Vet. Parasitol., 18:* 167-182.

NANTULYA, V. M.; DOYLE, J. J. & JENNI, L., 1976. Studies on *Trypanosoma (Nannomonas) congolense.* I. On the morphological appearance of the parasite in the mouse. *Acta Trop., 35:* 329-337.

ORMEROD, W. E., 1979. Development of Trypanosoma brucei in the Mammalian Host. p. 339-393 In W. H. R. Lumsden & D. A. Evans (eds) *Biology of the Kinetoplastida,* Vol. 2, Academic Press, New York.

ORMEROD, W. E.; VENKATESAN, S. & CARPENTER, R. G., 1974. The effect of immune inhibition on pleomorphism in *Trypanosoma brucei rhodesiense. Parasitology, 68:* 335-367.

OVERATH, P.; CZICHOS, J.; STOCK, V. & NONNENGAESSER, C., 1983. Repression of glycoprotein synthesis and release of surface coat during transformation of *Trypanosoma brucei. Embo. J., 2:* 1721-1728.

PIFANO, F., 1954. Nueva trypanosomiasis humana de la region neotropica producida por el *Trypanosoma rangeli,* con especial referencia a Venezuela. *Arch. Venez. Patol. Trop. Parasitol. Med., 2:* 89-120.

PIFANO, F.; MAYER, M.; ROMER, M.; DOMINGUEZ, E.; MEDINA, R. & BENAIM, E., 1949. Estado actual de las investigaciones en Venezuela sobre una nueva trypanosomiasis humana de la region neotropica producida por el *Trypanosoma rangeli. Arch. Venez. Patol. Trop. Parasitol. Med., 1:* 135-152.

PIZZI, T., 1953. Sobre el problema de las formas delgadas de *Trypanosoma cruzi. Bol. Invest. Parasitol., Chile, 8:* 26-30.

RIBEIRO, R. D.; NETO, F. M.; PRADO, J. C. & ALBUQUERQUE, S., 1986. Comportamento de formas sanguicolas do *Trypanosoma cruzi* obtidas de camundongos utilizados como filtros biologicos em cultura e em Triatomineo. *Rev. Saúde Publ. São Paulo, 20:* 129-132.

SCORZA, C.; URDANETA-MORALES, S. & TEJERO, F., 1986. *Trypanosoma (Herpetosoma) rangeli* Tejera, 1920: Preliminary report on histopathology in experimentally infected mice. *Rev. Inst. Med. Trop. São Paulo, 28:* 371-378.

STEINDEL, M.; CARVALHO PINTO, J. C.; TOMA, H. K.; MANGIA, R. H. R.; RIBEIRO-RODRIGUEZ, R. & ROMANHA, A. J., 1991. *Trypanosoma rangeli* (Tejera, 1920) isolated from a sylvatic rodent *(Echimys dasythrix)* in Santa Catarina Island, Santa Catarina State: first report of this trypanosome in southern Brazil. *Mem. Inst. Oswaldo Cruz, 86:* 73-79.

TEJERO, F.; GALLI, N. & URDANETA-MORALES, S., 1988. *Trypanosoma (Herpetosoma) rangeli* Tejera, 1920: Influence of host weight, size of inoculum, and route of infection upon experimental parasitemia. *Rev. Soc. Brasil. Med. Trop., 21:* 135-138.

TORREALBA, J. F.; PIFANO, F. & ROMER, M., 1950. Investigaciones sobre Enfermedad de Chagas y Trypanosomiasis rangeli en el Distrito Roscio, Estado Guarico, Venezuela. *Gaceta Med. Caracas, 58:* 85-98.

TOVAR, D.; URDANETA-MORALES, S. & TEJERO, F., 1989. *Trypanosoma (Herpetosoma) rangeli* Tejera, 1920: Study of the effects of the parasite on the vector. *Acta Cient. Venez., 40:* 208-214.

URDANETA-MORALES, S., 1983. Pleomorphism in trypomastigotes of *Trypanosoma cruzi* from blood and cell culture. *Tropenmed. Parasit., 34:* 225-228.

URDANETA-MORALES, S. & TEJERO, F., 1985. *Trypanosoma (Herpetosoma) rangeli* Tejera, 1920:

Mouse model for high, sustained parasitemia. *J. Parasitol., 71:* 409-414.

URDANETA-MORALES, S. & TEJERO, F., 1986. *Trypanosoma (Herpetosoma) rangeli* Tejera, 1920: Intracellular amastigote stages of reproduction in white mice. *Rev. Inst. Med. Trop. São Paulo, 28:* 166-169.

VICKERMAN, K., 1985. Developmental cycles and biology of pathogenic trypanosomes. *Brit. Med. Bull., 41:* 105-114.

WALTON, B. C. & SOUSA, C. E., 1967. Trypanosomes of the lesser anteater *Tamandua tetradactyla,* from Panama. *J. Parasitol., 53:* 956-961.

WIJERS, D. J. & WILLET, K. C., 1960. Factors that may influence the infection rate of *Glossina palpalis* with *Trypanosoma gambiense.* II. The number and morphology of the trypanosomes present in the blood of the host at the time of the infected feed. *Ann. Trop. Med. Parasitol., 54:* 341-350.