

AN UNUSUAL TRYPANOSOME IN *CEBUS GRISEUS* F. CUVIER, 1819, FROM COLOMBIA, SOUTH AMERICA

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SUMMARY

Small numbers of an unusual trypanosome were found by direct examination of smears made from peripheral blood of a single *Cebus griseus* monkey from Colombia, South America. No other infections with this parasite have been found in 46 *Cebus* monkeys. A very light infection with *T. minasense* was also observed in the *Cebus griseus* under study.

Though somewhat similar to *Trypanosoma conorrhini*, as described by various Authors and observed in experimental infections in mice during the present study, the parasite showed sufficient morphological and biological differences to exclude synonymy.

Blood cultures and subcultures developed in six different media. Under these conditions, the organisms remained mainly in leptomonad form.

No development occurred in the following insects when blood-meals were offered on the infected *Cebus* monkey: *Cimex lectularium*, *Triatoma protracta*, *T. infestans*, *T. barberi*, and *Rhodnius prolixus*.

Using culture material, no infections could be induced in one-week old white mice, one-week old white rats, golden hamsters, C3H mice, two *Cebus apella* and one *Cercopithecus aethiops*. No infections followed inoculation of arterial blood from the natural host into C3H mice, one-week old mice or a squirrel monkey.

The possibility of a mutant strain is considered, deriving from a trypanosome species of possible *T. conorrhini* or *T. rangeli*-like origin as an accidental development in an abnormal host.

INTRODUCTION

Primate trypanosomes in the Neotropical Region fall into two major groups: those resembling *Trypanosoma cruzi* Chagas 1909 or *Trypanosoma rangeli* Tejera 1920. Aside from *T. cruzi*, more than a dozen trypanosome species resembling *T. rangeli* have been described in the past from man and from non-human primates.

In the course of a series of surveys for blood parasites in South American monkeys,

one animal, a *Cebus griseus**, was found to harbor an unusual trypanosome. Of a total of 46 *Cebus* surveyed (27 *C. apella*, 16 *C. albifrons* and 3 *C. griseus*), only the fore-mentioned specimen of *C. griseus*, which received the identification number CAP5, was

* The nomenclature of *Cebus griseus* is subject to controversy. See W. C. O. Hill, "Primates", Vol. IV/A: Cebidae, pages xxi and 427-429.

This investigation was supported in part by Public Health Service Research Grant AI 04189-02 from the ICMRT Program, Office of International Research, National Institute of Health, U.S. Public Health Service.

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found infected with the trypanosome described here, while six other *Cebus* were infected with *T. minasense*, a *T. rangeli*-like species. The trypanosomes encountered during the surveys fell within the range of variation of either of the two established trypanosome groups, with the exception of those found in CAP5, which were markedly different from any previously described species.

In the genus *Cebus* the following trypanosomes have been reported: *Trypanosoma cebus*: FLOCH & ABONNENC^{8, 9, 11, 12} studied trypanosomes from *Cebus fulvus*. They obtained development both in triatomids and in experimental animals. The *Cebus fulvus* parasite, after differentiation from *T. devei*, *T. manguinhense* and *T. florestali*, was named *Trypanosoma cebus*.

Trypanosoma ariarii: GROOT, RENJIFO-SALCEDO & URIBE¹⁴ reported human infections with *T. ariarii*. A similar trypanosome was found in a *Cebus fatuellus* (= *C. apella*) kept as a pet in the house where the human infections were discovered. This trypanosome developed in *Rhodnius prolixus* and in baby mice (GROOT¹⁵).

Trypanosoma diasi: DEANE & MARTINS² described *T. diasi* from *Cebus apella apella*. The parasite developed in *Panstrongylus megistus*, *Triatoma infestans* and *R. prolixus*. They failed to infect mice, guinea-pigs and dogs.

MATERIAL AND METHODS

Cebus griseus. This monkey, imported to San Francisco by an animal importing firm (DUNN et al.⁶), was purchased, given the identification number CAP5, and kept in captivity at the University of California Medical Center animal vivarium for 18 months, after which the animal died from unknown causes **.

** International importing regulations require that primates exported from yellow fever areas be held in mosquito-proof cages for at least nine days before shipment. This regulation, and the fact that after this quarantine the monkeys are transported by air as soon as possible to San Francisco, makes it possible to postulate that the parasites during these surveys were probably acquired before time of capture and can therefore be considered natural infections.

Cultures.

a) *CAP5 trypanosome*. The CAP5 trypanosome was cultured several times from citrated blood from the *Cebus* monkey and maintained by subsequent subculturing. Cultures and subcultures grew at room temperature on the following media: autoclaved Little-Subbarow, non-autoclaved Little-Subbarow, modified Wenyon's, modified Wenyon's with urea (STEINERT²²), Tobie's diphasic broth with Locke's overlay and with Hank's solution, and Bacto-Tryptose.

b) *T. conorrhini*. In order to investigate the possibility of relationship, a strain of *T. conorrhini* was studied, isolated from wild *Triatoma rubrofasciata* from Singapore ***. In addition to passages in white mice, the strain was also cultured.

Invertebrates. Seventeen *Triatoma protracta*, 15 *T. infestans*, 11 *T. barberi*, 2 *Rhodnius prolixus* and 24 *Cimex lectularius* were allowed to feed on *Cebus* CAP5 during various periods. One group of bugs was kept at room temperature (about 23°C) and another at higher temperatures. After the feedings, droppings of the bugs were collected and examined microscopically. Dejecta were examined up to 70 days after the first feeding for most bugs, in some cases up to 256 days. The bugs were later carefully dissected and examined.

Vertebrates. Fifty-three white one-week old mice, 4 C3H-strain mice, 10 one-week old white rats and 2 golden hamsters were inoculated intraperitoneally or subcutaneously with culture fluid bearing leptomonads at various stages of development. Culture material was also given orally to the 2 hamsters. Two baby mice, 4 C3H-strain mice and one squirrel monkey (*Saimiri sciureus*) were inoculated intraperitoneally directly with arterial blood from CAP5.

In later experiments, use was made of mice kept from 4 to 8 days on a Vitamin B-complex-free diet before inoculation and during the time of observation. In this series,

*** These wild *T. rubrofasciata* were collected and sent from Singapore through the kindness of Dr. R. Desowitz, a courtesy which is gratefully acknowledged here.

altogether 30 Vitamin B-complex-free mice were inoculated with CAP5 culture, 32 normally fed mice serving as controls.

Numbers of organisms inoculated ran from 3×10^7 to 12×10^9 .

In experiments using *T. conorrhini* cultures, 16 mice on a normal diet and 37 on a Vitamin B-complex-free diet were inoculated.

In a new series of experiments, 2 *Cebus apella* were inoculated with CAP5 culture, once I.P., once I.M. and once I.V., and one *Cercopithecus aethiops*, an African monkey, was inoculated I.V.

Blood and tissue impression smears. Thick blood films were stained with Giemsa, thin films with May-Grünwald and Giemsa. Tissue impression smears were made from organs from 42 mice used in these experiments and from the squirrel monkey (after it died from unknown causes).

Postmortem tissue sections of CAP5. Heart, lung, liver, kidney, brain, spleen and muscle sections were fixed in Carnoy's and stained in Giemsa to allow microscopical examination.

Bloodform flagellates measurements. Measurements of the CAP5 flagellates as seen in the peripheral blood were performed under a compound microscope (1,000 \times magnification).

RESULTS

The parasite count in CAP5 was always very low, 4 being the maximum number of trypanosomes in one thick film. No division forms were ever seen in the blood. The infection was, however, perpetuated at a regular though low level, as indicated by the presence of trypanosomes in the bloodstream of the *Cebus* throughout the 18 months of observation.

From 66 thick and 51 thin bloodfilms the unusual trypanosome was seen 33 times in thick and 10 times in thin smears. In addition, the total of 117 films showed 16 other trypanosomes, identified as *T. minasense*.

The monkey was also infected with small numbers of an unidentified microfilaria.

Marked differences had been observed previously in morphology and size of microfilaria, according to the staining method and whether parasites were seen in thin or thick preparation (DUNN & LAMBRECHT⁷).

In this study significant morphological differences were also observed between trypanosomes in thick and those in thin film preparations.

In *thick films*, the trypanosomes from CAP5 have a nearly straight or slightly curved body, seldom conspicuously sinuous like most *T. rangeli*-like trypanosomes. About half the specimens show a sharp bend at about one third from the anterior end; at this point a small vacuole is sometimes observed. The posterior portion of the body is long and drawn-out. The anterior extremity tapers to a fine point. The average over-all length, including the free flagellum is 46.4 μ (range 42-56 μ); the free flagellum is 13.6 μ long; the width of the body at the nucleus 1.8 μ . The nucleus is rather small and oval-shaped. The kinetoplast, rounded or sometimes bean-shaped, occupies half to full width of the body at that point. The flagellum forms a few widely spaced undulations. In some specimens the anterior part of the body is covered with deep red, coarse granules. Chief identifying characteristics in both thick and thin smears include:

- a. The anterior part of the body stains a dark, intense blue, gradually fading into a lighter blue towards the anterior tip. The posterior part stains a pale blue, with a reticulate or even vacuolated protoplasm. A clear area is observed between nucleus and dark anterior portion of the body.
- b. Constriction of the body at the nucleus is a very characteristic feature. The constriction is enhanced by a body bulge found immediately beyond this point.
- c. Distance between kinetoplast and nucleus is extremely short.

In *thin films*, the trypanosomes appear much broader and somewhat longer. In many specimens, the side with the undulating membrane is strongly indented and fol-

lows the same undulations as the membrane. The posterior portion of the body is broader in thin than in thick preparations, ending in a sharply curved point or, in some cases, in a blunt stump. The marginal kinetoplast is generally smaller than that seen in thick films. Coarse, deep-red granules cover the dark anterior portion of the body of some specimens, as in thick preparations. The nucleus occupies the entire width of the body and has a band-like appearance; the constriction of the body at that point, although noticeable, is less pronounced than in thick smears. In all specimens a clear area can be seen between nucleus and dark anterior part of the body. An area of small vacuoles is common in the vicinity of the kinetoplast; in two specimens this area was also covered with red granules. In both thick and thin preparations, especially in the latter, the dark anterior portion of the body shows a marked longitudinally striated pattern.

Measurements of the CAP5 flagellates as seen in the peripheral blood of the original host are given in Table I.

As for the CAP5 trypanosome cultures and subcultures in the above mentioned nutrient media, development was most satisfactory in Tobie's medium and equally successful whether human blood or rabbit blood was used for the solid part of the medium, or when Locke's or Hank's solution was used as the overlaying liquid phase. The forms that developed were leptomonads and crithidia, forming random patterns of two to maybe ten short and somewhat stumpy organisms, the outward pointing flagella waving in slow motion. Growth is slow; large populations do not develop, but some cultures were still viable three months after the last transfer. When subcultured more frequently (weekly), somewhat larger populations develop in which larger clusters can be found, with 30 or more leptomonads. However, these clusters are never organized in a regular rosette pattern, so characteristic of the *T. conorrhini* cultures. The latter developed rather quickly and large populations occur. Groups of 50, or more, long, slender leptomonad forms in rosette pattern as characteristic. The organisms are extremely active, with hundreds of rapidly swimming forms as well as the rosettes. All stages of development are to be seen. These char-

acteristics serve to differentiate the cultures readily upon microscopic examination.

The few *T. minasense*-like trypanosomes seen in smears of peripheral blood of CAP5 apparently did not survive in culture. No *T. rangeli* or *T. cruzi*-like flagellates were observed in the cultures. Furthermore, none of the animals later inoculated with this culture material demonstrated these organisms. It was later demonstrated that when blood from a *Cebus apella*, showing only *T. minasense*, was cultured, only *T. rangeli*-like flagellates developed. No clusters were seen of short leptomonads, only numerous, independent, long, slender, rapidly undulating crithidial forms, typical of *T. rangeli*-group trypanosomes. Such slender forms were never observed in CAP5 cultures. It therefore appears reasonably certain that the flagellates here described do not belong to the *T. rangeli* or *T. cruzi*-like groups, but are indeed the culture form of an unrelated species.

On the other hand, the *T. conorrhini* forms developing in culture were very distinct from those developing from arterial blood from CAP5 in similar media. Inoculation of cultured *T. conorrhini* and of gut contents of the *T. rubrofasciata* into mice was successful. This provided additional evidence of biological distinction from the CAP5 trypanosomes, which has thus far failed to infect mice. Blood smears from *T. conorrhini*-infected mice showed important morphological differences from the CAP5 smears. These differences, as seen in thin film preparations, can be summarized as follows:

CAP5 trypanosomes

1. Long, broad, only slightly curved; posterior portion of body often broad or stumpy.
2. Cytoplasm behind nucleus staining markedly deep blue in Giemsa; anterior portion stains less intensely. Conspicuous constriction of body in nuclear region.
3. Nucleus broad, band or girdle-like.
4. Cytoplasm in darkly staining portion often with striated pattern.
5. Undulating membrane lies alongside one side of body.
6. End of free flagellum unfrayed, not swollen terminally.
7. Parabasal body inconspicuous.

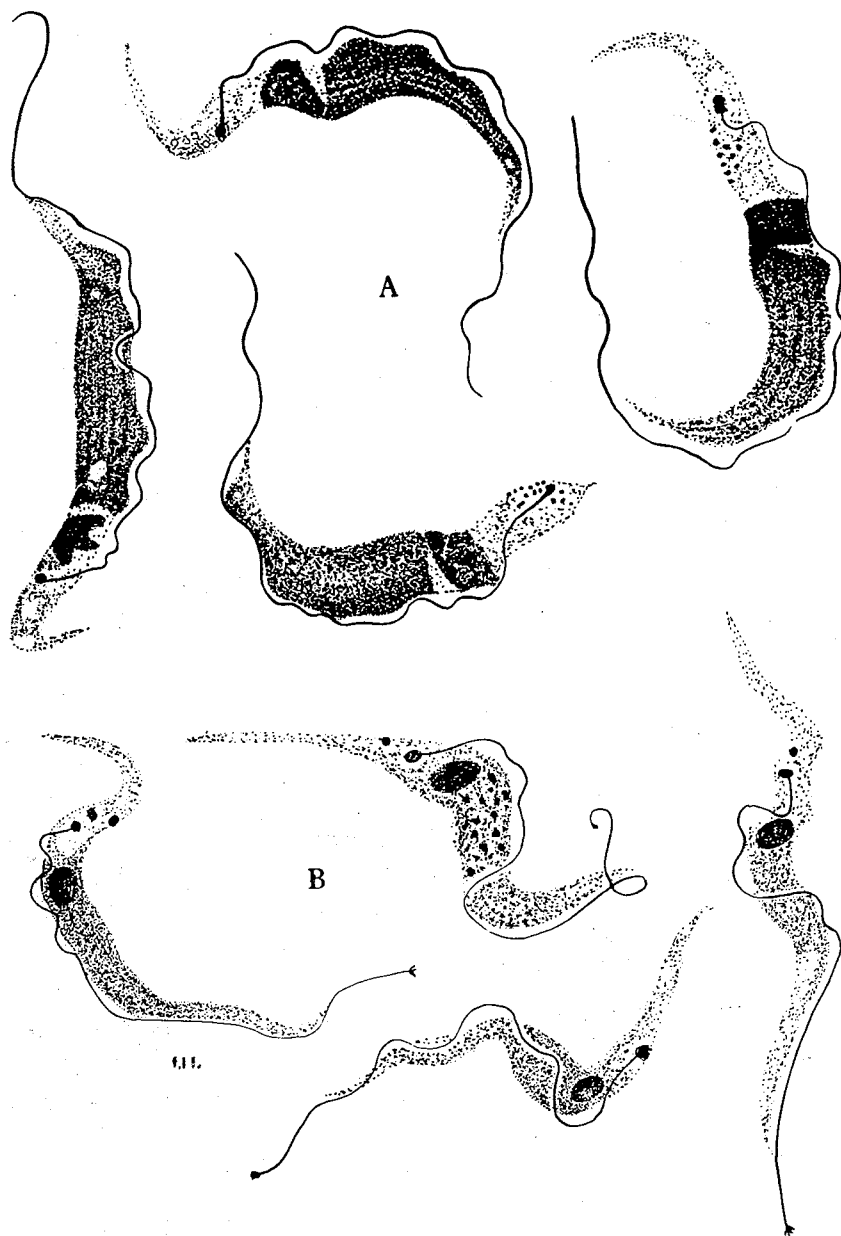


Fig. 1 — A) Trypanosomes observed in thin blood smears from the *Cebus* monkey CAP5. B) *T. conorrhini* observed in thin blood smears from experimental infections in white mice (1,000 \times).

T. conorrhini

1. Short, slender, sinuous; posterior end drawn out and narrow.
2. Anterior part of body darker than posterior, but less marked than in CAP5. No constriction of body as in CAP5 trypanosomes.

3. Nucleus small, oval-shaped lies "free" within the cytoplasm.
4. Not observed.
5. Undulating membrane often crosses body.
6. End of free flagellum frayed or with a stumpy terminal structure.
7. Parabasal body sometimes very prominent.

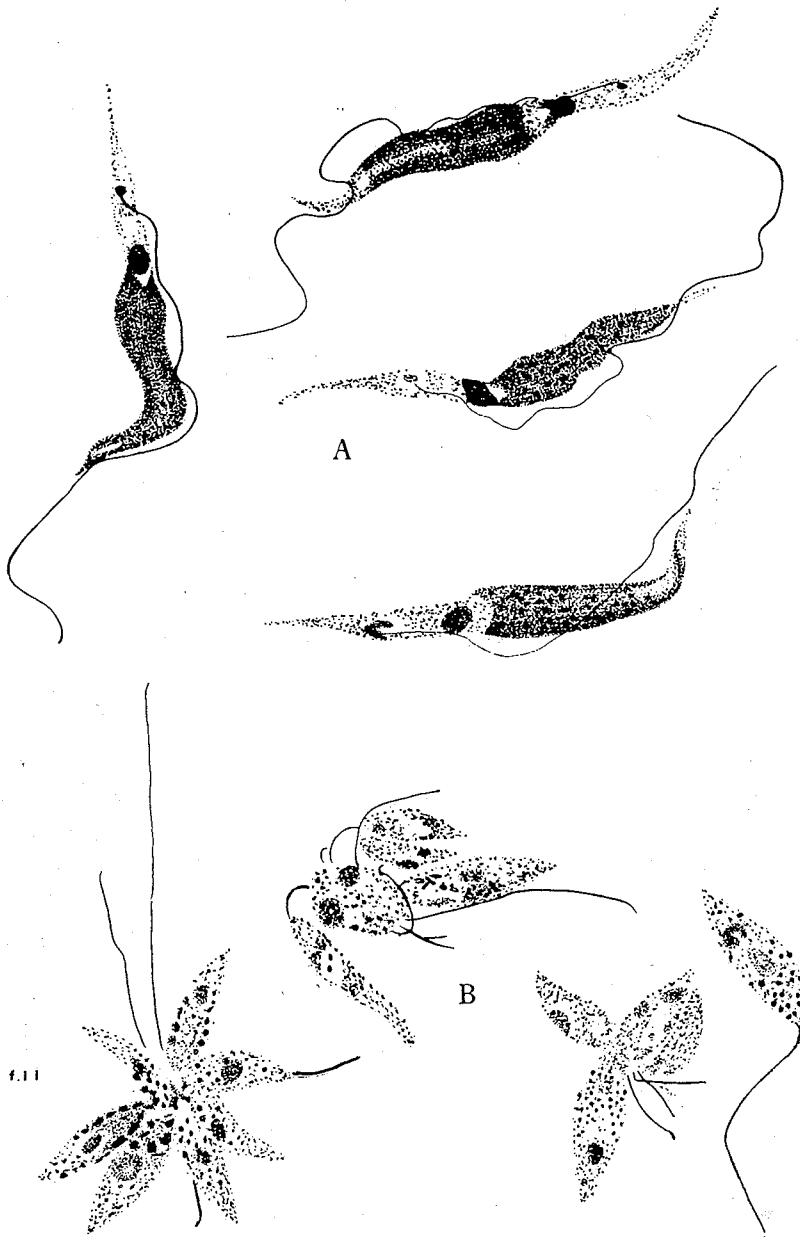


Fig. 2 — A) Trypanosomes observed in thick blood smears from the *Cebus* monkey CAP5. B) Example of clusters, and one single form, seen in a typical culture of CAP5 trypanosomes (1,000 \times).

T. conorrhini measurements are summarized in Table II.

Behaviour in invertebrates.

No flagellates were ever found in the dejecta or in the intestinal contents of the dissected bugs.

Behaviour in vertebrates.

No infections developed in any of the inoculated animals and none of the tissue impression smears made from organs from 42 mice and one squirrel-monkey, showed development of trypanosomes.

While the Vitamin B-complex-free diet before inoculation and during the time of observation seemed to increase somewhat the infection rate of *T. conorrhini*-inoculated mice, it did not promote infection with the CAP5 cultures. Of the 30 Vitamin B-complex-free mice inoculated with CAP5 culture and 32 normally fed control mice, none became infected.

In contrast to this, in experiments using *T. conorrhini* cultures, 44% of 16 mice on

a normal diet and 54% of 37 mice on a Vitamin B-complex-free diet, became infected.

No development occurred in the 2 *Cebus apella* inoculated I.P., I.M. and I.V. with CAP5 cultures as well as in one *Cercopithecus aethiops* inoculated I.V.

In the postmortem heart, lung, liver, kidney, brain, spleen and muscle tissue sections of CAP5, no trace of the parasite was found.

TABLE I

Measurements of the CAP5 trypanosomes from the bloodstream

	Thin smears: 10 trypanosomes		Thick smears: 30 trypanosomes	
	Range (μ)	Average (μ)	Range (μ)	Average (μ)
Total length (1)	42-57	50.9	42-56	46.4
Distance "A" (2)	4-10	8.5	6-10	8.6
Distance "B" (3)	4-7	5.5	4-7	5.4
Distance "C" (4)	16-29	23.7	13-24	18.8
Distance "D" (5)	11-17	13.2	10-17	13.6
Width (at nucleus)	3-6.5	4.0	1-2	1.8
R ₁ (6)	—	1.54	—	1.58
R ₂ (7)	—	0.60	—	0.75

(1) Total length: including free flagellum.

(2) Distance "A": from posterior tip to middle of kinetoplast.

(3) Distance "B": middle of kinetoplast to middle of nucleus.

(4) Distance "C": middle of nucleus to anterior tip.

(5) Distance "D" length of free flagellum.

(6) Ratio 1: $\frac{\text{distance posterior tip to kinetoplast}}{\text{distance mid-nucleus to kinetoplast}}$

(7) Ratio 2: $\frac{\text{distance posterior tip to mid-nucleus}}{\text{distance anterior tip to mid-nucleus}}$

TABLE II

Measurements of *T. conorrhini* (in μ) in experimental infections in white mice as reported by various Authors

Same measurements used as in Table I	LAMBRECHT Thin smears 10 trypanosomes	LAMBRECHT Thick smears 30 trypanosomes	MORISHITA	LAFONT	DIAS & CAMPOS SEABRA	FLOCH
Total length	41-46	30-44	50.03	28-42	40-41.2	23.0-39.0
Distance "A"	7-14	5-10	11.4	2.8-9.6	6.7-9.1	6.25
Distance "B"	4-5	3-5	3.7	1.4-3.5	3.0-4.2	4.25
Distance "C"	17-25	15-22	25.2	—	17.0-20.7	15.50
Distance "D"	6-9	6-10	7.5	4.2-9.8	9.1-10.9	6.0
Width, at nucleus ..	2.5-4.0	1.0-3.5	1.88	2.8	—	—
R ₁	2.16	1.82	3.0	5.0	2.2	1.4
R ₂	1.46	1.75	0.6	—	0.6	0.7

DISCUSSION

By default of finding a way to classify the unusual trypanosome from CAP5, it would seem permissible to propose a new species name. Unfortunately, failing development in both invertebrate and vertebrate hosts, we have little information about this parasite, except for its morphological aspect in the blood of one *Cebus*, and its rather strange behaviour in culture.

There are some certain resemblances between the CAP5 trypanosomes and *T. conorrhini* of various Authors (DONOVAN⁵; LAFONT^{16, 17}; SHORT & SWAMINATH²¹; MORISHITA²⁰; MALAMOS¹⁹; BONNE¹; DIAS & CAMPOS SEABRA⁴; FLOCH & ABONNENC¹⁰; GOSH & BISWAS¹³; LANGUILLON¹⁸; DEANE & DEANE³).

It is quite obvious from the measurements reported by various Authors, summarized in Table II, that *T. conorrhini* is a very polymorphic species. This fact was also commented upon by MORISHITA²⁰ in his detailed monograph, stating that this trypanosome in experimental infections varied considerably in staining characteristics as well as in size, being much smaller at the time of its

first appearance in the bloodstream. It would seem that the extensive polymorphic characters of *T. conorrhini* could be used as an argument in assigning the CAP5 trypanosomes as an aberrant form of that species. Biological evidence discussed in the preceding sections would seem to indicate, however, the CAP5 trypanosomes to be distinct from *T. conorrhini*.

The study of the parasite would suggest the possibility of its being a mutant form, possibly an aberrant variety of *T. conorrhini*, or one of the *T. rangeli*-like so common in South American monkeys.

Though *T. conorrhini* is a common parasite in *Triatoma rubrofasciata*, itself widely distributed in the tropics, natural infections in vertebrates have been reported only twice, both times in rats and by xenodiagnosis. Once in Java (BONNE¹), the second time in the State of Pará, Brazil (DEANE & DEANE³).

For the moment it seems advisable to publish these observations as they stand, until the parasite is seen again as a natural infection in a vertebrate, when complementary studies may reveal its true identity.

RESUMO

Trypanosoma incomum, encontrado em *Cebus griseus* F. Cuvier, 1819, da Colômbia, América do Sul

Trypanosoma incomum foi encontrado em pequeno número, ao exame direto de esfregaços de sangue periférico de um único macaco *Cebus griseus*, da Colômbia, América do Sul, num lote de 46 macacos *Cebus* examinados. Observou-se também ligeira infecção com *T. minasense* no exemplar de *Cebus griseus* estudado.

Embora assemelhando-se de certa forma ao *Trypanosoma conorrhini*, segundo descrevem alguns Autores e como se observou em infecções experimentais de camundongos durante a presente experiência, o parasita apresentou diferenças morfológicas e biológicas suficientes para excluir sinonímia.

Hemoculturas e subseqüentes repiques foram possíveis em seis diferentes meios nutrientes. Nestas condições, os organismos permaneciam principalmente sob a forma de leptômonas.

Não houve desenvolvimento do flagelado nos seguintes insetos alimentados no exemplar de *Cebus* infetado: *C. lectularium*, *T. protracta*, *T. infestans*, *T. barberi* e *R. prolixus*.

Utilizando como inóculo as formas culturais, não se conseguiu induzir infecção em camundongos e ratos albinos de uma semana de idade, "hamsters" dourados, camundongos da linhagem C3H, dois *Cebus apella* e um *Cercopithecus aethiops*. O inóculo de sangue arterial do hospedeiro encontrado naturalmente infetado com o tripanosoma em camundongos C3H, camundongos albinos de uma semana de idade e em um "macaco de cheiro" (*Saimiri sciureus*), também não produziu infecção.

A possibilidade de se tratar de uma linhagem mutante, derivada de uma espécie de tripanosoma de origem comum à do *T. conorrhini* ou do *T. rangeli* e que se teria desenvolvido em hospedeiro acidental, é considerada.

ACKNOWLEDGMENTS

I wish to extend my sincere appreciation to Dr. W. E. Greer and Mr. S. Gluck of Asiatic Animal Imports, Inc. for their collaboration, and to Drs. F. L. Dunn and D. Heyneman of the Hooper Foundation for their constructive criticism.

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Recebido para publicação em 2/2/1965.