SHORT COMMUNICATIONS

Trypomastigote Dimorphism and Satellite Deoxyribonucleic Acid in a Clone of *Trypanosoma (Schizotrypanum) dionisii*

By J. R. BAKER, A. J. LISTON AND LINDA F. SELDEN

*M.R.C. Biochemical Parasitology Unit, Molteno Institute, Downing Street,
Cambridge CB2 3EE*

(Received 24 June 1976)

INTRODUCTION

Trypanosoma (Schizotrypanum) dionisii Bettencourt & França, 1905, a parasite of Microchiroptera (insectivorous bats) of Europe and North Africa, develops *in vitro* mainly as epimastigotes and trypomastigotes, the latter being of two morphological forms – long, thin or short, broad individuals (Baker *et al.*, 1972). Determination of the buoyant density of the parasites' deoxyribonucleic acid (DNA) by Newton & Burnett (1972) revealed a satellite, which was heavier than the main DNA component, of unknown location in the organism; densities of 1.700 and 1.712 g ml⁻¹ were reported for the kinetoplasmic and nuclear DNA respectively (means of four determinations; standard deviations ± 0.001 g ml⁻¹). The possibility existed that the occurrence of two forms of trypomastigotes and the extra DNA component resulted from the presence of a mixed population of two species or subspecies in the strain (P3) being studied. This possibility has now been excluded by the examination of a clone derived *in vitro* from a single organism of this strain. A preliminary account has been published (Liston & Baker, 1976).

METHODS

Strain P3 of *T.(S). dionisii* was isolated from *Pipistrellus pipistrellus* (Baker & Thompson, 1971) and is routinely maintained *in vitro* at 28 °C in liquid culture medium L4NHS, derived from L4N (Baker *et al.*, 1972) by halving the serum and erythrocyte lysate content.

A clone was prepared as follows. Single small drops of medium from 3- to 4-day-old cultures of strain P3 in L4NHS medium, diluted with medium so that they contained about one organism, were placed (using a 1 ml disposable tuberculin syringe fitted with a 26 gauge 0.5 in hypodermic needle from which the bevelled end had been cut off and the tip ground smooth) on sterile 9 × 9 mm coverslips, each in a sterile plastic Petri dish (35 mm diam., 10 mm deep; Falcon no. 1008; Becton Dickinson, Wembley, Middlesex) containing a drop of sterile water for humidification. Each drop of medium was covered with a second similar coverslip; if the drop extended to an edge of the coverslip, it was discarded, as the resulting meniscus might have concealed a trypanosome. Suitable preparations, still within the Petri dish, were examined with a Wild M40 inverted microscope (Leitz, Park Street, Luton) at a magnification of $\times 150$ using phase-contrast illumination. Any drop thought to contain a single trypanosome was examined by two other observers. If all agreed that only one organism was present, the coverslips and 'sandwiched' drop were removed from the dish and transferred to 4N culture medium (Taylor & Baker, 1968), modified by replacing the liquid overlay with sterile filtrate (Millipore; 0.22 μ m pore size) from a 3- to 6-day-old L4NHS

culture of the same strain (an uninoculated bottle of each batch of modified medium was incubated at 28 °C to check its sterility and the absence of trypanosomes). The inoculated medium 4N, contained in either 28 ml McCartney bottles (5 ml base; 1 ml overlay) or 7 ml Universal bottles (1 ml base; 0.2 ml overlay), was incubated at 28 °C. Cultures were examined after 1 and 2 weeks: if any trypanosomes were seen, some medium was sub-inoculated to unmodified 4N medium. All procedures except microscopical examination were done aseptically in a sterile cabinet that had previously been u.v. irradiated.

Trypomastigotes were separated from L4NHS cultures by the method of Nogueira, Bianco & Cohn (1975), modified by incubating the initial suspension for 20 min in preserved guinea-pig serum (Wellcome) diluted in isotonic saline to the equivalent of a 1:10 serum dilution. The suspension was then centrifuged (3000 g; 15 min), resuspended in medium 199 (Wellcome) containing 1 % (v/v) heat-inactivated calf serum (Wellcome no. 1), and passed down a column (about 150 × 5 mm) of sterile glass ballotini (grade 12). The column was eluted with resuspension medium; epimastigotes lysed by the guinea-pig serum were retained, only trypomastigotes (of both types) being eluted. By selecting suitable cultures, suspensions containing a high proportion of long, thin trypomastigotes could be obtained.

DNA was extracted, dialysed and ultracentrifuged in caesium chloride to determine its buoyant density (Newton & Burnett, 1972).

Culture fluid containing trypanosomes was spread thinly on microscope slides, air-dried, fixed with methanol and stained with Giemsa stain for morphological examination.

RESULTS

Ten single parasites were isolated and inoculated into modified 4N culture medium. A limited amount of multiplication occurred with four of these isolates, but only one (no. 3) was successfully established; it was passaged after 6 days into unmodified 4N medium and transferred 8 days later to L4NHS, in which it was maintained at 28 °C. Stabilates were prepared after one and two passages in L4NHS (MIC118 and MIC119 respectively) and stored in liquid nitrogen. (MIC designates stabilates in J. R. Baker's collection at Molteno Institute, Cambridge.)

Two determinations of DNA buoyant density were made on extracts from morphologically mixed populations (predominantly epimastigotes) at dilutions corresponding to 1×10^8 and 2×10^8 organisms ml⁻¹; one was made on a suspension corresponding to 1.5×10^8 organisms ml⁻¹ of trypomastigotes, separated as described above, containing 95 % (144/151) long, thin trypomastigotes and 5 % short trypomastigotes. Densitometer tracings (Fig. 1) revealed the heavy satellite component (1.717 g ml⁻¹), fast banding, kinetoplastic (1.700 g ml⁻¹ in uncloned material, 1.699 in the clone) and nuclear components (1.712 g ml⁻¹ in uncloned material, 1.711 g ml⁻¹ in the clone). There was no significant difference between any of these values, nor between them and the mean of four determinations on uncloned material given by Newton & Burnett (1972).

Cultures of the clone in both liquid and diphasic blood-nutrient agar media (4N) grew initially as epimastigotes. Later, trypomastigotes developed that were of the two forms described and illustrated earlier (Baker *et al.*, 1972) as 'long, thin forms and shorter broader individuals'. Occasional sphaeromastigotes were seen.

DISCUSSION

The production of two forms of trypomastigotes *in vitro*, the possession of the heavy satellite 'C' DNA component by this cloned line of strain P3 and by one morphological form of it (the long, slender trypomastigotes), and the similarity of the DNA densities,

Fig. 1. Microdensitometer tracings of deoxyribonucleic acid (DNA) bands obtained by analytical ultracentrifugation in caesium chloride after extraction by the method of Newton & Burnett (1972): (a) DNA from original isolate of *T. dionisii*, strain P3; (b) DNA from clone 3 of strain P3 (morphologically mixed population, mainly epimastigotes); (c) DNA from a preparation of 95 % long, thin trypomastigotes and 5 % short trypomastigotes of clone 3. C, Satellite component; K, fast banding, kinetoplastic component; N, nuclear component; S, standard *Micrococcus lysodeikticus* DNA (buoyant density 1.7310 g ml^{-1}).

show conclusively that these features of *T. (S.) dionisii* are characters of the specific genotype and not the result of the isolates being composed of more than one strain or species. Thus the separation of this species from the other member of the subgenus recorded from Microchiroptera in Europe, *T. (S.) vespertilionis* Battaglia, 1904, is upheld. Trypomastigote dimorphism and satellite DNA have both been seen in three isolates of *T. dionisii* from British *P. pipistrellus* and in one from Belgium. Neither has been seen in two isolates of *T. vespertilionis* from *Nyctalus noctula* and seven from *P. pipistrellus* in Britain, including one clone (Green, 1974). Several isolates of the subgenus *Schizotrypanum* from South American bats have DNA buoyant densities different from those of *T. dionisii* or *T. vespertilionis* in Europe (J. R. Baker, unpublished results) and work is in progress to characterize them further.

REFERENCES

- BAKER, J. R. & THOMPSON, G. B. (1971). Two species of *Trypanosoma* from British Chiroptera (Bats). *Transactions of the Royal Society of Tropical Medicine and Hygiene* **65**, 427.
- BAKER, J. R., GREEN, S. M., CHALONER, L. A. & GABORAK, M. (1972). *Trypanosoma (Schizotrypanum) dionisii* of *Pipistrellus pipistrellus* (Chiroptera): intra- and extracellular development *in vitro*. *Parasitology* **65**, 251–263.
- GREEN, S. M. (1974). A method for aseptic production of cloned cultures of trypanosomes *in vitro*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **68**, 269.
- LISTON, A. J. & BAKER, J. R. (1976). Trypomastigote dimorphism and satellite DNA in a clone of *Trypanosoma (Schizotrypanum) dionisii*. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **70**, (in the Press).
- NEWTON, B. A. & BURNETT, J. K. (1972). DNA of Kinetoplastidae: a comparative study. In *Comparative Biochemistry of Parasites*, pp. 185–198. Edited by H. Van den Bossche. New York: Academic Press.
- NOGUEIRA, N., BIANCO, C. & COHN, Z. (1975). Studies on the selective lysis and purification of *Trypanosoma cruzi*. *Journal of Experimental Medicine* **142**, 224–229.
- TAYLOR, A. E. R. & BAKER, J. R. (1968). *The Cultivation of Parasites in vitro*. Oxford and Edinburgh: Blackwell Scientific Publications.