KINETOPLAST AND OTHER SATELLITE DNAs OF KINETOPLASTIC AND DYSKINETOPLASTIC STRAINS OF *TRYPANOSOMA*

HARMUT C. RENGER and DAVID R. WOLSTENHOLME. From the Division of Biology, Kansas State University, Manhattan, Kansas 66502. Dr. Renger's present address is the Department of Pathology, New York University School of Medicine, New York 10016. Dr. Wolstenholme's present address is the Department of Biology, University of Utah, Salt Lake City, Utah 84112

INTRODUCTION

Members of the protozoan order Kinetoplastida (Honigberg et al., 1964), which includes the genera *Trypanosoma, Leishmania,* and *Crithidia,* are characterized by the presence of a body known as a kinetoplast. The kinetoplast is a modified region of a mitochondrion (Meyer et al., 1958; Steinert, 1960; Clark and Wallace, 1960) which contains so much DNA that it can be detected by Feulgen staining (Bresslau and Scremin, 1924).

DNA isolated from a kinetoplast fraction of *Trypanosoma lewisi* was found to have a buoyant density less than that of the cell's nuclear DNA and to comprise mainly covalently closed, circular molecules 0.4 μ in contour length, and some noncircular molecules (Renger and Wolstenholme, 1970). A proportion of the circular DNA was in the form of catenanes in which many circles were apparently interlocked with one or more adjacent circles. A DNA separated from whole cells of *Trypanosoma cruzi,* and presumed to be kinetoplast DNA, had similar properties (Riou and Paoletti, 1967; Riou and Delain, 1968). Also, circular molecules 0.74 μ long were found to be included in DNA from a kinetoplast fraction of *Trypanosoma mega* (Laurent and Steinert, 1970).

Loss of kinetoplast DNA from cells of a number of species of Kinetoplastida following treatment with diphenylamine compounds or with one of a number of basic dyes has been reported (Werbitzki, 1910; Mühlpfordt, 1959, 1963 *a,* 1963 *b,* 1964; Trager and Rudzinska, 1964; Guttman and Eisenman, 1965; Steinert and Van Assel, 1967; Simpson, 1968; Riou, 1968; Renger and Wolstenholme, 1970). Such organisms are usually referred to as dyskinetoplastic (Trager and Rudzinska, 1964). Dyskinetoplastic cells of in vitro culture strains cannot be maintained indefinitely. However, a few species of trypanosomes, all members of the so called *brucei* group (Hoare, 1964), can still be maintained in the blood of the vertebrate host after apparent induced loss of their kinetoplast

DNA (for a review see Mühlpfordt, 1964). Two dyskinetoplastic strains, *Trypanosoma equiperdum* and *Trypanosoma equinum,* are also known which apparently originated from "spontaneous mutants" of kinetoplastic cells (Tobie, 1951; Hoare, 1954).

In view of our findings concerning *T. lewisi* we considered it of interest to make a study of the buoyant densities and form and structure of the DNAs of these two dyskinetoplastic strains, together with the DNAs of the kinetoplastic strain of *T. equiperdum* and the kinetoplastic species, *Trypanosoma congolense. T. congolense* is not a member of the *brucei* group.

MATERIAL AND METHODS

Four vertebrate blood strains of trypanosomes were obtained from the American Type Cell Culture Collection (Rockville, Md.): the kinetoplastic strain of *Trypanosoma equiperdum* (ATCC No. 30019), the dyskinetoplastic strain of *T. equiperdum* (ATCC No. 30023), dyskinetoplastic *T. equinum* (ATCC No. 30029), and kinetoplastic *T. congolense* (ATCC No. 30018). The dyskinetoplastic strain of *T. equiperdum* originated from an organism found in blood infected with normal kinetoplastic *T. equiperdum* organisms (Tobie, 1951). The dyskinetoplastic strain *T. equinum* is thought to be a mutant of the kinetoplastic *Trybanosoma evansi* (Hoare, 1954).

All trypanosomes were maintained in albino rats (weighing 100–300 g each) by syringe passage every 2 days. Infected blood was obtained by cardiac puncture and the parasites were isolated by differential centrifugation as described previously (Renger and Wolstenholme, 1970). Details of all other preparative techniques were exactly as described previously for *Trypanosoma lewisi* (Renger and Wolstenholme, 1970). These were preparation of kinetoplast fractions, and of crude lysates of kinetoplasts and whole cells for both analytical cesium chloride density gradient centrifugation and electron microscopy; DNA purification; analytical CsCl density gradient centrifugation; preparative CsCl, and CsCl–ethidium bromide density gradient centrifugation; preparation of thin sections and of DNA-protein monolayers for electron microscopy.
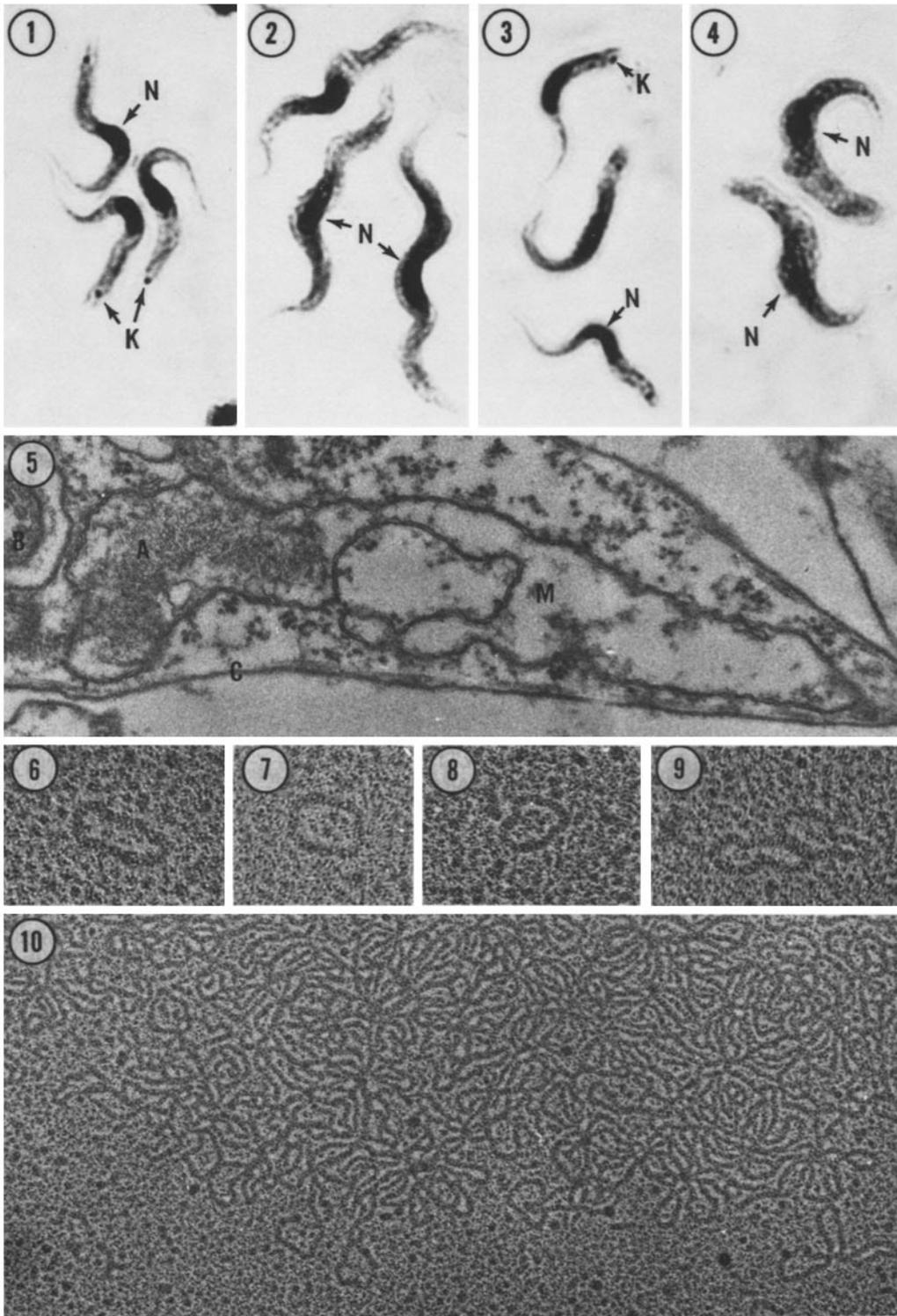
Electron micrographs were made with a Hitachi HU-11B electron microscope (using projector pole piece 2) at an original magnification of 11,000, calibrated with a diffraction grating replica (2160 lines/ mm). Measurements of molecules were made on positive prints at a magnification of 250,000.

Smears of trypanosomes were fixed in acetic acid: ethanol (1:3), stained with Giemsa's, mounted in immersion oil, and examined by bright-field illumination in a Zeiss Photomicroscope with a 100 × apochromatic oil immersion objective. Micrographs were made on Adox KB14 film (FR corporation).

RESULTS

Light microscopy of Giemsa-stained trypanosomes (Figs. 1–4) confirmed the presence or absence of kinetoplasts in cells of the four strains. A single kinetoplast could be seen at the posterior tip of each cell of the kinetoplastic strain of Trypanosoma equiperdum and of Trypanosoma congolense. No such stained body could be discerned in cells of the dyskinetoplastic strain of Trypanosoma equiperdum or of Trypanosoma equinum.

In thin sections of cells of Trypanosoma lewisi a mass of 25–30-A fibrils situated within an enlarged portion of a usual cristae-bearing mitochondrial vesicle has been shown to represent the kinetoplast DNA (Ris, 1962; Mühlpfordt, 1963 a, 1963 b). A similar mass of fibrils was found in longitudinal sections of cells of the kinetoplastic strain of T. equiperdum but the vesicle surrounding the fibrils, which often extended anteriorly, was usually devoid of cristae (Fig. 5). Absence or poor development of mitochondrial cristae has been shown to

be a general characteristic of trypanosomes of the brucei group which are maintained in the blood of laboratory vertebrates (Mühlpfordt, 1963 a, 1963 b). Vesicles similar to those found in the kinetoplastic organisms were seen in sections of dyskinetoplastic T. equiperdum cells but the mass of DNA-containing fibrils was never located.

The cesium chloride density gradient equilibrium band positions of DNA from whole cells of the four strains are shown in Fig. 11. Most of the DNA extracted from cells of each of the strains banded at a density of 1.708 g/cm³. A light DNA component at $\rho = 1.702$ was indicated by a shoulder on the main band of each of the T. equiperdum strains and of the T. equinum strain.

DNA from each of the strains included a distinct light satellite at $\rho = 1.692$ for the two T. equiperdum strains, $\rho = 1.694$ for T. equinum, and $\rho = 1.695$ for T. congolense. The light satellite DNA accounted for 12% of the total DNA of the two T. equiperdum strains and for 14% and 23% respectively of the total DNAs of T. equinum and T. congolense.

In rotary shadowed electron microscope preparations, DNA from whole cells of each of the strains consisted mainly of long linear molecules. In DNA from the kinetoplastic strain of T. equiperdum and from T. congolense, circular molecules were also found (Figs. 6–8). The mean length of the circles from T. equiperdum was 0.31 µ and from T. congolense 0.27 µ (Fig. 12). As some variation has been found in mean contour length of circular DNA molecules from a single DNA sample in dif-

Figures 1–4 Bright-field light micrographs of Giemsa-stained cells of trypanosomes. Nuclei (N) can be seen in all the cells. A kinetoplast (K) is clearly visible in each cell of the kinetoplastic strain of Trypanosoma equiperdum (Fig. 1) and of Trypanosoma congolense (Fig. 3). Cells of the dyskinetoplastic strain of Trypanosoma equiperdum (Fig. 2) and of Trypanosoma equinum (Fig. 4) lack a kinetoplast. All four micrographs, × 2200.

Figure 5 An electron micrograph of a longitudinal section through a cell of the kinetoplastic strain of Trypanosoma equiperdum showing the kinetoplast consisting of a mass of DNA-containing fibrils (A) within an enlarged portion of a mitochondrion (M). In this strain the mitochondria lack the usual cristae. A basal body (B) lies next to the kinetoplast. C, cell wall. × 49,000.

Figures 6–10 Electron micrographs of rotary shadowed molecules of DNA.

Figures 6 and 7 Single circular molecules 0.3 µ in contour length from a kinetoplast fraction of Trypanosoma equiperdum. Both micrographs, × 110,000.

Figure 8 A single circular molecule 0.25 µ in contour length from whole cell DNA of Trypanosoma congolense. × 110,000.

Figure 9 Two apparently interlocked 0.3 µ circular molecules from a kinetoplast fraction of Trypanosoma equiperdum. × 110,000.

Figure 10 A mass of DNA apparently made up of interlocking 0.3 µ circules from a kinetoplast fraction of Trypanosoma equiperdum. × 67,000.

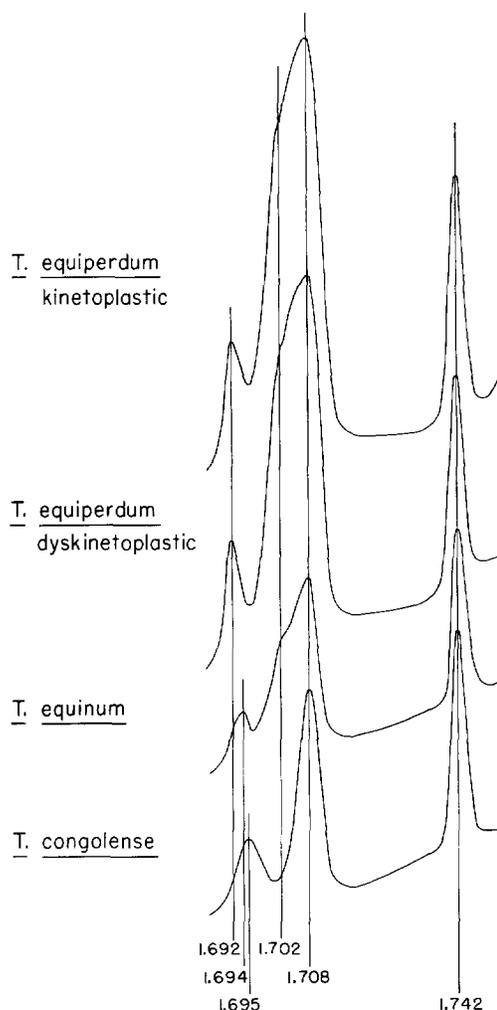Neither single circular molecules nor catenanes were found in DNA from dyskinetoplastic *T. equiperdum* or *T. equinum*. Also, in DNA from these strains, there was no indication of a population of noncircular molecules with a homogeneous length similar to that of the circles in DNA from kinetoplastic cells. These results indicate, therefore, that the light satellite DNA, at least of the dyskinetoplastic strains, comprises linear molecules.

Further experiments were conducted to elucidate the relationship of the light satellite DNA, the small circular molecules, and the kinetoplast DNA of kinetoplastic strains. A kinetoplast fraction was prepared from cells of kinetoplastic *T. equiperdum* and treated with DNase to remove nuclear DNA contamination (Renger and Wolstenholme, 1970). Examination in the electron microscope of thin sections of a pellet of this fraction revealed mainly double unit membrane-limited vesicles and kinetoplasts. DNA in a lysate of this fraction banded exclusively at the same density as the light satellite of whole cell DNA (Fig. 13) In the electron microscope, the kinetoplast DNA appeared as 0.3 μ circular molecules and catenanes. Long, often noncircular molecules (up to at least 10 μ in length) were occasionally found associated with the catenanes. A few circular molecules were found which were two or three times larger than the 0.3 μ circles. Also, molecules apparently consisting of two or three 0.3 μ interlocked circles were seen (Fig. 9).

A sample of kinetoplast DNA was heated at 100°C for 5 min, quenched in ice, and centrifuged to equilibrium in a CsCl gradient. A single band was formed at a density only 4 mg/cm³ greater than native density (Fig. 13). This result is similar to that of a similar experiment concerning *T. lewisi* ciruclar kinetoplast DNA (Renger and Wolstenholme, 1970) and is in agreement with most of the kinetoplast DNA being in the form of circular molecules which are covalently closed (Vinograd and Lebowitz, 1966). In such a molecule all of the phosphodiester bonds of each polynucleotide chain are intact. Upon heating they show resistance to denaturation (Vinograd and Lebowitz, 1966; Nass, 1969; Wolstenholme, Kirschner, and Gross, unpublished; Wolstenholme and Renger, 1970), and even if hydrogen bond separation occurs the two chains cannot separate from each other due to their topological bonding. Upon cooling they "snap back" into the native configuration (Dawid and Wolstenholme, 1967).

FIGURE 11 Microdensitometer tracings of ultraviolet photographs of cesium chloride buoyant density gradients of DNA from whole cells of the four strains of trypanosomes indicated. The reference band (1.742) to the right is native DNA of SPO1.

ferent protein monolayer preparations (Wolstenholme and Dawid, 1968), it remains possible that the lengths of the circles in the two strains are in fact the same. In DNA from both of these strains a few large masses were also seen (Fig. 10), the appearance of which was consistent with their comprising interlocked small circular molecules. Evidence supporting this interpretation has been presented for similar structures found in DNA from *T. lewisi* (Renger and Wolstenholme, 1970) and they are therefore referred to as catenanes.
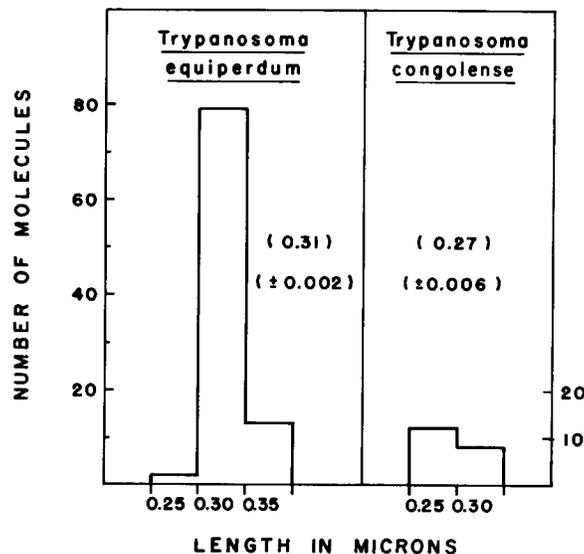
FIGURE 12   Frequency distribution of lengths of circular molecules of whole cell DNA of kinetoplastic *Trypanosoma equiperdum* and *Trypanosoma congolense*. The mean and standard error of each sample are given.

Confirmation of this interpretation was obtained by examining the heated and quenched kineto-plast DNA in the electron microscope. Many apparently double-stranded, 0.3 μ circular molecules were seen which were undistinguishable from native circular molecules. A number of catenanes, also apparently comprising double-stranded DNA molecules, were also found. (Under the conditions used to make these preparations, single-stranded DNA either collapses or appears as kinky threads, poor in contrast and therefore easily distinguished from double-stranded DNA.) (For further discussion see Dawid and Wolstenholme, 1968.)

Whole cell DNA from each of the four strains was heated and quenched, and centrifuged to equilibrium in CsCl gradients. The results are shown in Fig. 14. The peak of the main band of DNA of all strains increased by 18–20 mg/ml, indicating that most of the DNA had been extensively denatured. DNA from each strain included a band at $\rho = 1.709$–1.712, which is what would be expected if the light satellite DNAs (native $\rho = 1.692$–1.695, Fig. 11) had also been extensively denatured. Heated and quenched DNAs from kinetoplastic *T. equiperdum* and *T. congolense* also included a distinct small band (about 2% of the total absorption attributable to DNA) at $\rho = 1.696$ and $\rho = 1.697$, respectively. These positions are only 4 mg/cm³ and 2 mg/cm³ from

the position of native light satellite DNA of each strain. This result, together with the absence of such a satellite from DNA of dyskinetoplastic *T. equiperdum* and *T. equinum*, is consistent with these bands representing covalently closed, circular DNA from the two strains. This was again confirmed by examining the heated and quenched DNAs in the electron microscope. Apparently double-stranded circular molecules were found in the DNAs from the two kinetoplastic strains.

Taking into consideration that denatured DNA shows a hyperchromicity of approximately 30% compared to native DNA (Marmur and Doty, 1962), then the data in Fig. 14 indicate that about 80% of the light satellite DNA of each of the kinetoplastic strains is either in the form of circular molecules containing at least one phosphodiester bond break (open circles), or, like the light satellite DNA of the dyskinetoplastic strains, of linear molecules. In order to determine the form of this DNA, whole cell DNA of kinetoplastic *T. equiperdum* was centrifuged to equilibrium in a CsCl-ethidium bromide gradient. Examination with blue-violet light revealed a main fluorescent band and a smaller *less dense* band. Rotary shadowed DNA from each band was examined in the electron microscope, and found in each case to comprise long linear molecules. Since covalently closed circles bind less dye than open circular or linear

whole cell

kinetoplast

kinetoplast heated
and quenched
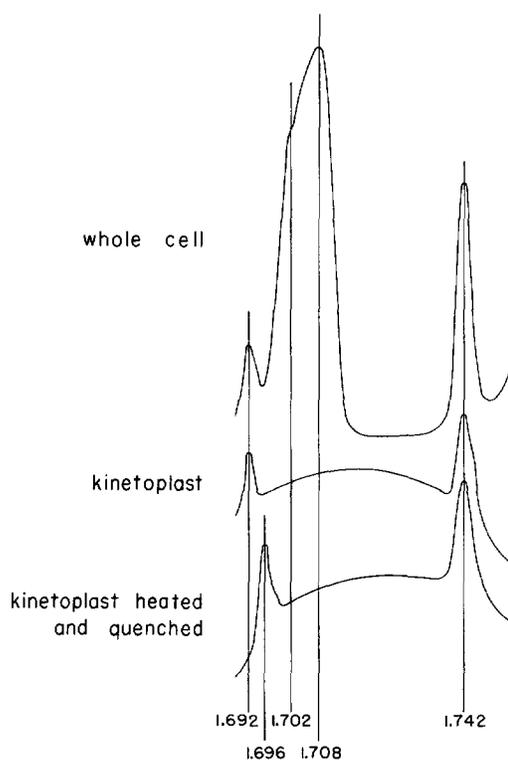
1.692 | 1.702   1.742

1.696 1.708

FIGURE 13 Microdensitometer tracings of ultraviolet photographs of cesium chloride buoyant density gradients of DNA from whole cells, and from a lysate of a kinetoplast fraction of kinetoplastic *Trypanosoma equiperdum*. The two upper tracings are of native DNA, the lower one of DNA which was heated at 100°C for 5 min and then quenched in ice. The reference band (1.742) to the right is native DNA of SPO1.

T. equiperdum
kinetoplastic

T. equiperdum
dyskinetoplastic

T. congolense

T. equinum

1.696 | 1.709   1.726   1.742
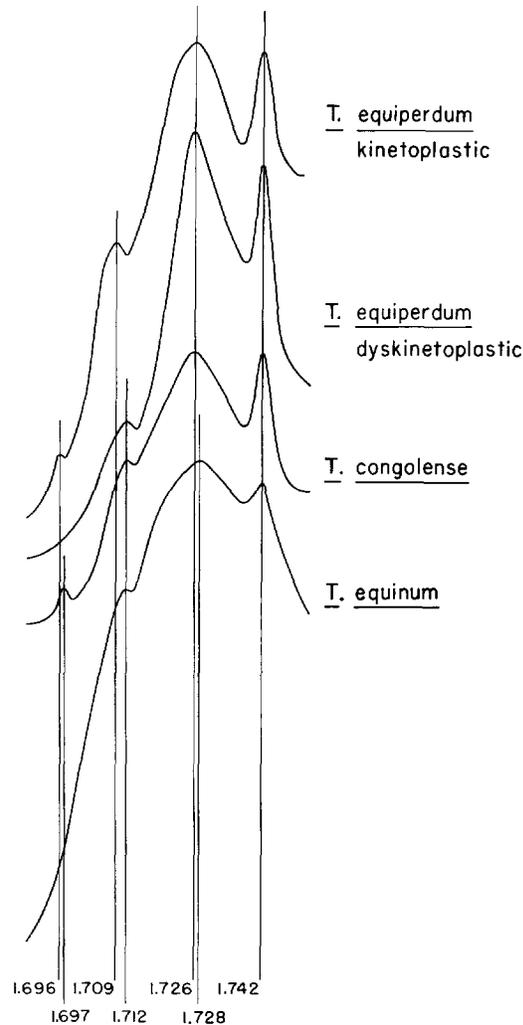
1.697   1.712   1.728

FIGURE 14 Microdensitometer tracings of ultraviolet photographs of cesium chloride buoyant density gradients of DNA from whole cells of the four strains of trypanosomes indicated. In each case the DNA was heated at 100°C for 5 min and then quenched in ice. The reference band (1.742) to the right is native DNA of SPO1.

DNA molecules, they band at a greater density (Radloff et al., 1967; Bauer and Vinograd, 1968). As no third lower band was visible in the present experiment, the amount of total DNA in the covalently closed form is indicated to be very low. At equilibrium in a CsCl–ethidium bromide gradient the relative positions of linear DNA molecules are expected to be, as in the absence of the dye, dependent upon their base composition (Bauer and Vinograd, 1968). Our observations therefore indicate that most of the light satellite DNA of kinetoplastic *T. equiperdum* comprises noncircular DNA molecules.

DISCUSSION

The results presented indicate that the kinetoplast DNA of kinetoplastic *Trypanosoma equiperdum*, like the kinetoplast DNA of *Trypanosoma lewisi* (Renger

and Wolstenholme, 1970), comprises mainly covalently closed, small, circular molecules, many of which are associated in catenanes.

It is clear from the data that the small circular molecules are found only in organisms which manifest a kinetoplast detectable by staining.

All of the kinetoplastic and dyskinetoplastic strains examined, however, possessed a light satellite DNA of the same buoyant density as the covalently closed circular kinetoplast DNA, but ap-

parently comprising noncircular molecules of greater length than the kinetoplast circles. In kinetoplastic cells the linear light satellite DNA is in an approximately four times greater concentration than the kinetoplast DNA. This is in contrast to the findings for *T. lewisi*. Almost all of the light satellite DNA of this species is in the form of small covalently closed circles (Renger and Wolstenholme, 1970).

The presently available data are consistent with the linear light satellite DNA being of nuclear origin; most of the kinetoplast DNA (at least in *T. equiperdum*) was indicated to be circular, and cytochemical examination did not reveal any concentrations of DNA other than the nucleus and the kinetoplasts.

Riou et al. (1966) reported that whole cell DNA which they extracted from *Trypanosoma equiperdum* comprised a main band at $\rho = 1.707$ and a lighter component at $\rho = 1.701$. The latter DNA corresponds more closely in buoyant density to the DNA which appeared as a shoulder on the main band of our extracts of *T. equiperdum* whole cell DNAs, rather than to the kinetoplast DNA or to the nonkinetoplast DNA of similar density of this species.

Evidence has been presented for the actual loss of kinetoplast DNA from the cell following treatment with certain drugs (Simpson, 1968; Steinert and Van Assel, 1967; Renger and Wolstenholme, 1970). However, whether or not the kinetoplast DNA has actually been lost from the cells in the two dyskinetoplastic strains used in the present study is not at all clear. Two alternative explanations are possible. Upon loss of circularity this DNA may have become so dispersed in the large mitochondrial complement that it is not detectable cytologically. Alternatively, loss of circularity may have accompanied transfer of this DNA to the nucleus. This latter suggestion was made originally by Werbitzki (1910). It is lent credibility by observations of apparent short-time fusion of kinetoplast and nucleus in a number of strains of trypanosomes. Electron micrographs of these fusions have been interpreted as demonstrating disappearance of separating membranes between the organelles (Chakraborty and Sanyal, 1962; Mühlpfordt, 1963 a, 1963 b).

The different buoyant densities of kinetoplast DNA (reviewed in Renger and Wolstenholme, 1970), and the different sizes of circular molecules reported for different trypanosomes (0.27 μ for *T.*

congolense; 0.31 μ for *T. equiperdum*; 0.4 μ for *T. lewisi* (Renger and Wolstenholme, 1970), 0.45 μ for *T. cruzi* (Riou and Delain, 1968), 0.74 μ for *T. mega* (Laurent and Steinert, 1970), suggests that the genetic information carried by kinetoplast DNA of the different species may vary both quantitatively and qualitatively.

REFERENCES

Bauer, W., and J. Vinograd. 1968. The intercalation of closed circular DNA with intercalative dyes. I. The superhelix density of SV40 DNA in the presence and absence of dye. *J. Mol. Biol.* 33:141.

Bresslau, E., and L. Scremin. 1924. Die Kerne der Trypanosomen und ihr Verhalten zur Nuklealreaktion. *Atch. Protistenk.* 55:509.

Chakraborty, J., and A. B. Sanyal. 1962. Some observations on the nucleus and the kinetoplast of *Leishmania donovani*—the kalazar parasite. *Proc. 5th Int. Congr. Electron Microsc.* 2:UU-8.

Clark, T. B., and F. G. Wallace. 1960. A comparative study of kinetoplast ultrastructure in the Trypanosomatidae. *J. Protozool.* 7:115.

Dawid, I. B., and D. R. Wolstenholme. 1967. Ultracentrifuge and electron microscope studies on the structure of mitochondrial DNA. *J. Mol. Biol.* 28:233.

Dawid, I. B., and D. R. Wolstenholme. 1968. Renaturation and hybridization studies of mitochondrial DNA. *Biophys. J.* 8:65.

Guttman, H. M., and R. N. Eisenman. 1965. Acriflavin-induced loss of kinetoplast deoxyribonucleic acid in *Crithidia fasciculata* (*Culex pipiens* strain). *Nature* (*London*). 207:1280.

Hoare, C. A. 1954. The loss of the kinetoplast in trypanosomes with special reference to *Trypanosoma evansi*. *J. Protozool.* 1:28.

Hoare, C. A. 1964. Morphology and taxonomic studies on mammalian trypanosomes. X. Revision of the systematics. *J. Protozool.* 11:200.

Honigberg, B. M., W. Balamuth, E. C. Bovee, J. O. Corliss, M. Godjics, R. P. Hall, R. R. Kudo, N. D. Levine, A. R. Loeblich, J. Weiser, and D. H. Wenrich. 1964. A revised classification of the phylum Protozoa. *J. Protozoa.* 11:7.

LAURENT, M., and M. STEINERT. 1970. Electron microscopy of kinetoplastic DNA from *Trypanosoma mega. Proc. Nat. Acad. Sci. U.S.A.* **66**:419.

MARMUR, J., and P. DOTY. 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J. Mol. Biol.* **5**:119.

MEYER, H., M. DE OLIVEIRA MUSACCHIO, and I. DE ANDRADE MENDONCA. 1958. Electron microscopic study of *Trypanosoma cruzi* in thin sections of infected tissue cultures and of blood-agar forms. *Parasitology.* **418**:1.

MÜHLPFORDT, H. 1959. Vergleichende Untersuchung über die Wirkung des Trypaflavins auf den Blepharoplast verschiedener Trypanosomenarten. *Z. Tropenmed.* **10**:19.

MÜHLPFORDT, H. 1963 *a.* Über die Bedeutung und Feinstruktur des Blepharoplasten bei parasitischen Flagellaten. I. Teil. *Z. Tropenmed. Parasitol.* **14**:357.

MÜHLPFORDT, H. 1963 *b.* Über die Bedeutung und Feinstrukture des Blepharoplasten bei Parasitischen Flagellaten. II. Teil. *Z. Tropenmed. Parasitol.* **14**:475.

MÜHLPFORDT, H. 1964. Über den Kinetoplasten der Flagellaten. *Z. Tropenmed. Parasitol.* **15**:289.

NASS, M. M. K. 1969. Mitochondrial DNA. II. Structure and physicochemical properties of isolated DNA. *J. Mol. Biol.* **42**:529.

RADLOFF, R., W. BAUER, and J. VINOGRAD. 1967. A dye-buoyant-density method for the detection and isolation of closed circular duplex DNA. *Proc. Nat. Acad. Sci. U. S. A.* **57**:1514.

RENGER, H. C., and D. R. WOLSTENHOLME. 1970. Kinetoplast DNA of the hemoflagellate *Trypanosoma lewisi. J. Cell Biol.* **47**:689.

RIOU, G. 1968. Disparition de l'ADN du kinetoplaste de *Trypanosoma cruzi* cultive au présence de bromure d'ethidium. *C. R. Acad. Sci. Ser. D.* **266**:250.

RIOU, G., and E. DELAIN. 1968. Electron microscopy of the circular kinetoplastic DNA from *Trypanosoma cruzi*: occurrence of catenated forms. *Proc. Nat. Acad. Sci. U. S. A.* **62**:210.

RIOU, G., and C. PAOLETTI. 1967. Preparation and properties of nuclear and satellite deoxyribonucleic acid of *Trypanosoma cruzi. J. Mol. Biol.* **28**:377.

RIOU, G., R. PAUTRIZEL, and C. PAOLETTI. 1966. Fractionnement et charactérisation de l'acide désoxyribonucléique (DNA) de trypanosome (*Trypanosoma equiperdum*). *C. R. Acad. Sci. Ser. D.* **262**:2376.

RIS, H. 1962. Ultrastructure of certain self-dependent cytoplasmic organelles. *Proc. 5th Int. Congr. Electron Microsc.* **2**:XX–1.

SIMPSON, L. 1968. Effect of acriflavin on the kinetoplast of *Leishmania tarentolae.* Mode of action and physiological correlates of the loss of kinetoplast DNA. *J. Cell Biol.* **37**:660.

STEINERT, M. 1960. Mitochondria associated with the kinetonucleus of *Trypanosoma mega. J. Biophys. Biochem. Cytol.* **8**:542.

STEINERT, M., and S. VAN ASSEL. 1967. The loss of kinetoplastic DNA in two species of Trypanosomatidae treated with acriflavin. *J. Cell Biol.* **34**:489.

TOBIE, E. J. 1951. Loss of the kinetoplast in a strain of *Trypanosoma equiperdum. Trans. Amer. Microsc. Soc.* **70**:251.

TRAGER, W., and M. A. RUDZINSKA. 1964. The riboflavin requirement and the effects of acriflavin on the fine structure of the kinetoplast of *Leishmania tarentolae. J. Protozool.* **11**:133.

VINOGRAD, J., and J. LEBOWITZ. 1966. Physical and topological properties of circular DNA. *J. Gen. Physiol.* **49**:103.

WERBITZKI, F. W. 1910. Über blepharoblastlose Trypanosomen. *Zentrobl. Bakteriol. Parasitenk. Infektionskr. Hyg. Abt. Orig.* **53**:303.

WOLSTENHOLME, D. R., and I. B. DAWID. 1968. A size difference between mitochondrial DNA molecules of urodele and anuran Amphibia. *J. Cell Biol.* **39**:222.