

# THE LOSS OF KINETOPLASTIC DNA IN TWO SPECIES OF TRYPANOSOMATIDAE TREATED WITH ACRIFLAVINE

M. STEINERT and SUZANNE VAN ASSEL

From Laboratoire de Morphologie Animale, Faculté des Sciences, Université Libre de Bruxelles, Belgium

## ABSTRACT

The effects of acriflavine on two species of Trypanosomatidae, *Crithidia luciliae* and *Trypanosoma mega*, have been investigated. It has been observed that kinetoplastic (i.e. mitochondrial) DNA is lost in a high percentage of acriflavine-treated cells. Resting flagellates, from stationary-phase or hemin-deficient cultures, are considerably more resistant to the acridine than are flagellates from a log-phase culture. When the kinetoplast has retained some DNA and still remains visible in stained smears, it appears reduced in size, and its ultrastructure is extremely abnormal: the DNA fibrils, clearly visible in normal kinetoplasts, are condensed; they appear as an electron-opaque, apparently homogeneous mass, separated from the membranes by a space of low electron-opacity. Analyses of DNA extracts, with high speed centrifugation in CsCl density gradients, revealed that the satellite band, presumably kinetoplastic DNA, is lost by trypanosomes grown for 5 days in the presence of acriflavine. Radioautography was used to study the effects of acriflavine on thymidine-<sup>3</sup>H incorporation in *C. luciliae*. At the concentration which affects the kinetoplast specifically, the dye produces an 87% inhibition of thymidine incorporation in this organelle. The kinetics of this inhibition suggest a direct effect on replication. No decrease in incorporation occurs in the nucleus. These results lead to the conclusion that loss of kinetoplastic DNA is due to continued growth and cell division in the absence of kinetoplastic DNA replication. Several hypotheses are discussed concerning the specificity of the dye's action upon the replication of extrachromosomal DNA.

The kinetoplast (1) of Trypanosomatidae is a self-duplicating body which is a differentiated part of the large, single mitochondrion found in these flagellates (4, 30, 32, 39). It stains intensely with the Feulgen reaction (3) and incorporates tritiated thymidine (29). Its DNA, which replicates in a cyclic way during the interphase (35), can be separated from nuclear DNA by CsCl gradient centrifugation (5, 6, 24). The kinetoplastic, i.e. mitochondrial, DNA of trypanosomes and related flagellates appears to be quite distinct from the nuclear DNA of the same species: its buoyant

density and melting temperature are lower and its capacity to "renature" is exceptionally high (6).

The hypothesis that such an extranuclear DNA might be involved in the coding of mitochondrial proteins has been formulated (31, 35), and some support for this idea is found in certain aspects of the biology of the more highly specialized trypanosomes, i.e. the species belonging to the *Trypanosoma brucei* group (40). Two distinct morphological forms, generally described as the culture and bloodstream forms, occur during their life cycle. The former has a well-developed mitochondrion

and active Krebs cycle enzymes; it oxidizes substrates through the cytochrome electron-transfer chain. The latter, whose mitochondrion is reduced in size and rather poor in cristae, appears to lack some of the mitochondrial enzymes and to use an alternative  $\alpha$ -glycerophosphate cycle for the re-oxidation of  $\text{NaDH}_2$  (8, 9). Similar cyclic changes probably occur in some species of the related genus *Leishmania* (14). Such a difference might be due to the repression and "derepression" of some inducible operon, as suggested by Gibor and Granick (7) in a general discussion of extrachromosomal inheritable systems. Evidence for the location of this hypothetical operon in the kinetoplast is suggested by the fact that *all* the "kinetoplastless" strains of trypanosomes are of the bloodstream type; they never revert to the culture form (19).

Apparently kinetoplastless forms can be induced in Trypanosomatidae by acridine dyes (see Mühlpfordt, reference 20, for a review of the subject). However, by thorough studies on the fine structure of these abnormal cells, Mühlpfordt (20) and Trager and Rudzinska (37) independently showed that the DNA contained in the kinetoplast is lost, but that the membranes are maintained. The latter authors therefore suggested that the term "dyskinetoplastic" should be used to designate these cells. This term will be used here to define any cell which shows no visible trace of kinetoplastic DNA. Ultimately, the dyskinetoplastic condition becomes lethal, except in the highly evolved trypanosomes of the *brucei* group mentioned above, which probably have acquired the ability to synthesize an alternative enzyme system, as a closer adaptation to parasitic life in the mammalian host.

In a series of experiments designed to get better insight into the structure and function of the kinetoplast, we have used acriflavine in order to obtain dyskinetoplastic cells of the less evolved species, *Crithidia luciliae* and *Trypanosoma mega*. It will be shown in this paper that both species may lose their kinetoplastic DNA as a result of acriflavine treatment, that such treatment specifically inhibits the synthesis of this particular DNA without interfering with nuclear DNA synthesis, and that kinetoplastic DNA is lost as a result of the disequilibrium affecting cell division and DNA replication.

#### MATERIALS AND METHODS

The two strains of *Trypanosoma mega* and *Crithidia luciliae* originated from the Institut pour la Recherche

Scientifique en Afrique Centrale, Lubumbashi, Congo Republic, and were cultivated in Boné's medium (2) at pH 7.4, 24°C, under very light rotatory agitation. Smears for light microscopy were fixed in methanol and stained with Giemsa's reagent. Acriflavine (The British Drug Houses Ltd., London) stock solutions were prepared shortly before use and sterilized by Millipore filtration. All cultures were protected from light.

#### Electron Microscopy

The fixation procedure of Ryter and Kellenberger (26), initially designed for the fixation of bacterial nuclei, was used without major modification, and was followed by uranyl-acetate staining. Embedding was done in Araldite. Silver and grey sections were used that eventually were stained in Reynold's lead solution (23). Electron micrographs were taken with a Hitachi HS 6 microscope at an original magnification of 10,000 $\times$ , or with an AEI model EM 6 B microscope, at original magnifications of 20,000 $\times$  or 30,000 $\times$ .

#### Thymidine- $^3\text{H}$ Incorporation by Acriflavine-Treated Cells

The experiments were principally designed to determine the effects of acriflavine on DNA synthesis in the kinetoplast as compared to the nucleus. 40  $\mu\text{g}/\text{ml}$  acriflavine were added to a growing culture of *Crithidia luciliae*. Aliquots of this culture were taken just before the addition of the dye and at different times thereafter; they were given a 15 min pulse of 50  $\mu\text{c}/\text{ml}$  thymidine- $^3\text{H}$  (The Radiochemical Centre, Amersham, England; 1.9 c/ml). This short incubation was stopped by mixing the cell suspension with nine volumes of ice-cold buffered isotonic saline solution containing 1 mg/ml carrier thymidine. The flagellates were centrifuged, washed in saline, recentrifuged, spread, and dried on gelatin-coated slides. Radioautographs of the smears were made in the usual fashion (34), with Ilford K<sub>2</sub> emulsion in gel form.

#### Isopycnic Centrifugation

*Trypanosoma mega* showed, in preliminary experiments, a higher peak of satellite DNA than did *C. luciliae* and was, for this reason, preferentially used for subsequent density gradient analysis. Total DNA was extracted according to the method of Ray and Hanawalt (22), slightly modified in that the 95% ethanol extractions were suppressed, the washed cells being immediately lysed in 1% dodecyl sodium sulfate. The final extract was analyzed in the Beckman G.2400 spectrophotometer and was found to contain 8-30  $\mu\text{g}$  DNA/ml. After adequate dilution, in order to obtain about 2  $\mu\text{g}$  DNA/ml, 1.25 g/ml optical grade CsCl was added and the samples were run for 20-24 hr at 44,770 rpm in a Beckman-Spinco model

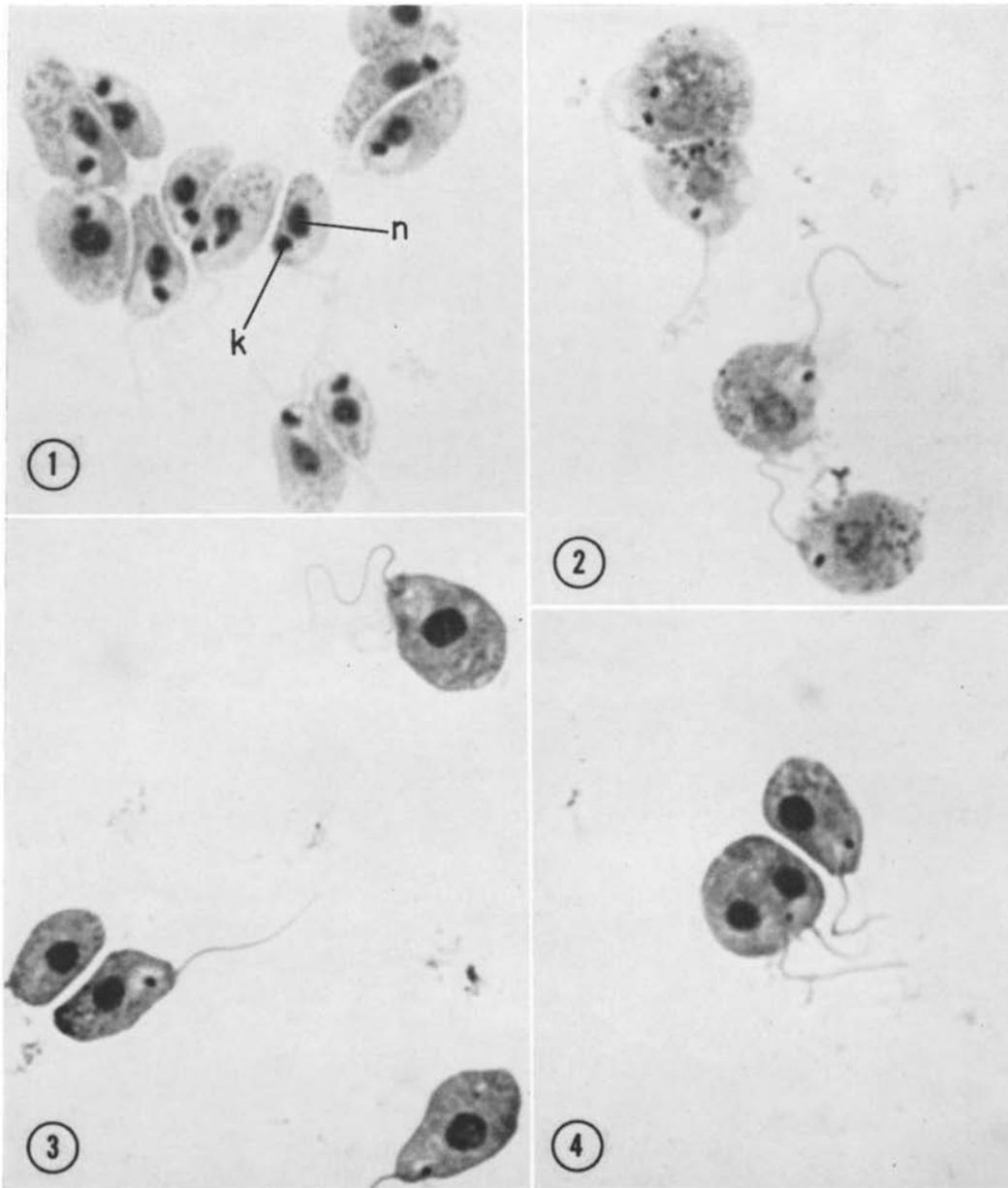


FIGURE 1 *Crithidia luciliae*. Control cells showing the normal size of the kinetoplast. *k*, kinetoplast; *n*, nucleus.  $\times 2,000$ .

FIGURE 2 *Crithidia luciliae*. Cells grown for 8 hr, 15 min in a medium containing 20  $\mu\text{g}/\text{ml}$  acriflavine. Most kinetoplasts are equally reduced in size.  $\times 2,000$ .

FIGURES 3-4 *Crithidia luciliae*. Individuals from a culture grown for 19 hr, 40 min in a medium containing 20  $\mu\text{g}/\text{ml}$  acriflavine. The kinetoplasts are much reduced in size or microscopically absent. Fig. 4 shows a dividing cell with duplicated flagella and nuclei, but with a single fragment of kinetoplast. If the division had been completed, one of the daughter cells would inevitably have been dyskinetoplastic.  $\times 2,000$ .

E analytical centrifuge. Photographs of the gradients were taken by the absorption method at  $260\text{ m}\mu$  and were scanned with a Joyce-Loebl MK IIIC microdensitometer (Joyce, Loebl & Co. Ltd., Gateshead-on-Tyne, England). Buoyant densities were calculated according to Sueoka's formula (36) and the CsCl gradient figures of Ifft et al. (11). Calculations were based on a reference DNA sample of *Micrococcus lysodeikticus* ( $\rho = 1.731$ ) (27).

DNase digestion was performed on an aliquot of the undiluted extract, after thorough dialysis in the cold against the buffer NaCl 0.1 M, Tris 0.01 M, MgCl<sub>2</sub> 0.01 M, at pH 7.9.

## RESULTS

The effects of acriflavine were found to be very similar in both species studied and, unless the species is specifically stated, the following results refer to both of them. *Trypanosoma mega* as well as *Crithidia luciliae* become dyskinetoplastic as a result of acriflavine treatment; the only obvious differ-

ence observed between the two species, in these experiments, lies in their sensitivity towards different concentrations of acriflavine; we found that the optimal concentrations for obtaining dyskinetoplasty are 2–4  $\mu\text{g}/\text{ml}$  for *T. mega* and 20–40  $\mu\text{g}/\text{ml}$  for *C. luciliae*.

A log-phase culture keeps on growing at a normal rate for a few generations after the addition of the dye. The kinetoplast divides at each cell bipartition through the usual process of lateral elongation and pinching off; but there is no concomitant growth, and this unbalanced process results in a stepwise decrease in the size of the organelle (Figs. 1–6). Unequal bipartition of the kinetoplast then becomes frequent, already beginning at the second division after the addition of acriflavine. It leads to the appearance of dyskinetoplastic cells (Fig. 4). In cultures of *Crithidia luciliae* treated with 20  $\mu\text{g}/\text{ml}$  acriflavine, the first dyskinetoplastic cells are found 12 hr after the addi-

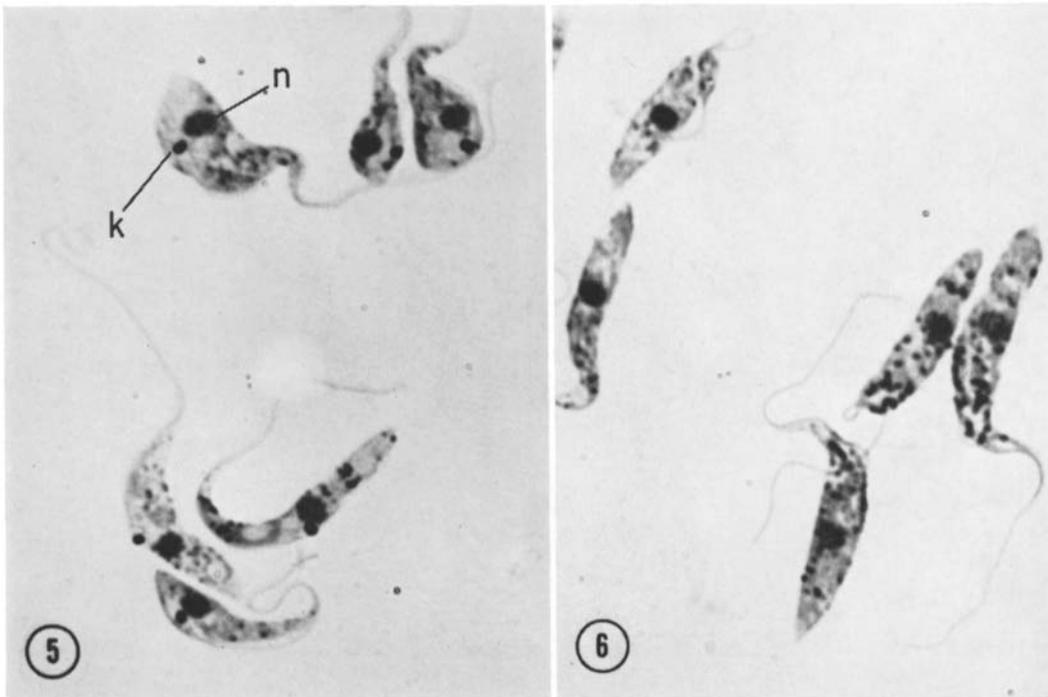


FIGURE 5 *Trypanosoma mega*. Normal control culture form. *k*, kinetoplast; *n*, nucleus.  $\times 2,000$ .

FIGURE 6 *Trypanosoma mega*. Culture form, grown for 4 days in a medium containing 2  $\mu\text{g}/\text{ml}$  acriflavine. Many cells are dyskinetoplastic; most of the others have a kinetoplast so reduced in size that it could easily be mistaken for one of the many cytoplasmic metachromatic granules, in this black and white photomicrograph.  $\times 2,000$ .

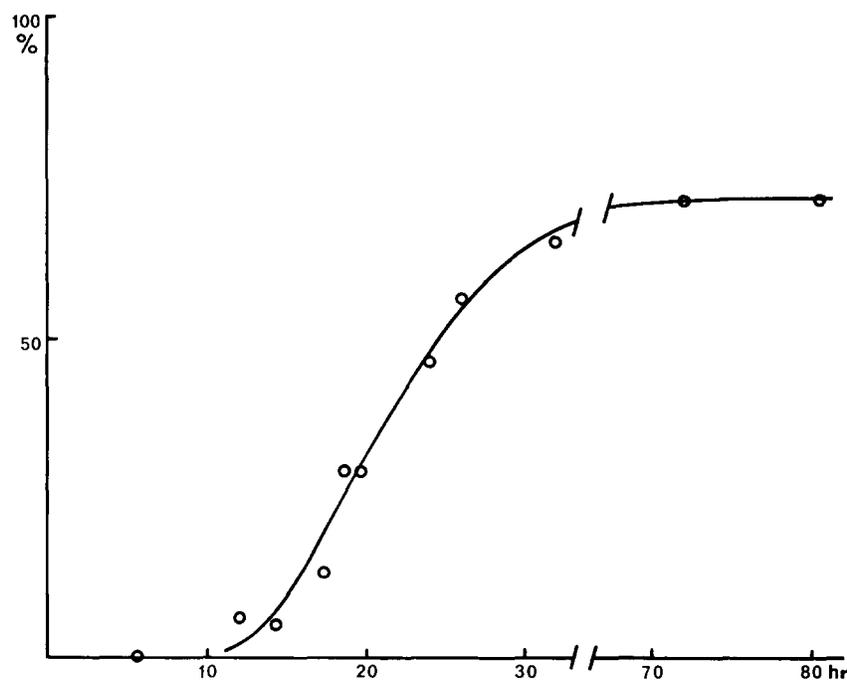


FIGURE 7 Appearance of dyskinetoplastic cells in growing cultures of *C. luciliae*, treated with 20  $\mu\text{g}/\text{ml}$  acriflavine for different lengths of time. The percentage of dyskinetoplastic cells is plotted against the incubation time in presence of acriflavine.

tion of the dye (Fig. 7); this length of time closely corresponds to two cell generations (the generation time is 6.2 hr for this species, in the medium and under the conditions defined in the Materials and Methods section).

When the incubation with acriflavine is continued, the percentage of dyskinetoplastic cells increases, and the growth rate of the acriflavine-treated cultures decreases progressively. However, this growth rate is difficult to follow accurately on the basis of cell counts: in both normal and treated cultures, a variable number of flagellates adhere to the glass walls of the culture flask; others form the so-called "rosettes", in which a large number of sister cells remain temporarily associated by posterior cytoplasmic bridges. When growth ceases, as many as 75% of the crithidia (Fig. 7) or trypanosomes may be dyskinetoplastic. After prolonged treatment with acriflavine, other abnormalities are found in addition to the apparent loss of the kinetoplast: the mean size of the cells becomes higher and giant polynucleate cells can frequently be observed, especially in *T. mega*, which often also shows shortened and/or additional flagella.

Increasing the concentrations of the dye above

the optimal range leads to toxicity and slows down the growth rate, but the increase may result in a decrease in the final yield of dyskinetoplastic cells. In fact, the efficiency of acriflavine treatment in producing dyskinetoplasty decreases as the growth rate of the culture slows down. Table I shows a quantitative estimation of kinetoplast alteration in two aliquots of the same culture of *C. luciliae*: one was treated with acriflavine in the mid log-phase; the second was treated at the end of the log-phase. Dyskinetoplasty could not be induced in stationary-phase cultures, and hemin deficiency, which inhibits growth, greatly limits the effects of acriflavine on the kinetoplast (Table II).

Electron microscopical observations were made on normal *Crithidia luciliae*, and on cells treated with 20  $\mu\text{g}/\text{ml}$  acriflavine for 24 or 72 hr. The kinetoplast of normal crithidia (Figs. 8-9) appears to be composed *entirely* of a fine fibrillar material, which is known to contain DNA. The fibrils stain strongly with uranyl acetate; they fill the whole kinetoplast and are probably in direct contact with the inner membrane of the double mitochondrial (or kinetoplastic) envelope. They usually seem to lie parallel to the main axis of the

TABLE I  
*Comparison of the Effects of Acriflavine upon the Kinetoplast of Mid and End Log-Phase Crithidia Luciliae*

Sample	Aspect of the kinetoplast		
	Normal	Reduced	Not visible
A	0	53	47.0
B	14.5	85	0.5

Aliquots of a growing culture were taken 24 and 48 hr after inoculation of the medium. The aliquots were incubated with 20  $\mu\text{g}/\text{ml}$  acriflavine for another period of 24 hr; this growth period corresponds, for the first sample (A), to the mid log-phase, and, for the second sample (B), to the end of the logarithmic phase. Giemsa-stained smears of each sample were examined microscopically to determine presence and size of kinetoplasts. The results are expressed in per cent of the total number of kinetoplasts counted in each sample.

organism. The width of these fibrils is difficult to measure with precision, owing to the coarseness of the Araldite background, but a value of 30 A is probably close to reality. A few mitochondrial cristae may be found at the point where the kinetoplast merges into the typical mitochondrial part of the complex. A zone of higher density is generally observed in the center of the kinetoplast (Fig. 9), probably because of denser packing of fibrils. Ribosomes or ribosome-like granules could not be observed with certainty either in the kinetoplast or in the mitochondrion.

Among the acriflavine-treated crithidia, no major difference was found between cells treated for 24 hr and those treated for 72 hr with the acridine dye, except for the final yield of dyskinetoplastic cells. The kinetoplast, in those crithidia which still retain one, is much smaller than usual and has lost its normal structure. Figs. 10-15 show different degrees of reduction in size affecting both the inner material and the membranes (Figs. 14-15). The DNA-containing fibrils are much less distinct than in the controls. More often, they cannot be observed at all; the contents of the kinetoplast look like a compact, homogeneous, electron-opaque mass. This DNA-containing material is constantly separated from the membranes by a zone of greatly reduced opacity; this abnormal aspect suggests a closer packing of the DNA fibrils and their detachment from the kinetoplast membrane. A typical mitochondrion is still present

(Figs. 12-15), but it is obviously less developed than in untreated crithidia. Mitochondrial cristae are frequently seen as rounded empty vesicles (Figs. 12-13). The nuclei seem to be quite normal, as does the cytoplasm which remains full of polysomes. The structure and extension of the endoplasmic reticulum do not seem to be altered.

Thymidine is actively incorporated into the nuclear and the kinetoplastic DNAs of untreated, growing crithidia. The percentage of labeled cells is a function of the relative duration of the replication phase, a matter which will be discussed in a following paper. Counts of silver grains over treated crithidia reveal a sharp decrease in the incorporation of thymidine into kinetoplastic DNA; this decrease occurs very shortly after the addition of 40  $\mu\text{g}/\text{ml}$  acriflavine, as shown in Fig. 16. A 30 min exposure to acriflavine brings about an 80% diminution in the uptake of label into the kinetoplast. Even after much longer treatments, however, a small residual incorporation of thymidine occurs, equivalent to about 13% of the normal DNA synthesis. The incorporation of thymidine into nuclear DNA is unaffected in the acridine-treated crithidia.

The analysis, in caesium chloride density gradient, of the total DNA extract from *T. mega* shows two components (Fig. 17): the nuclear component is found as a major band with a density of 1.703; a lighter, presumably kinetoplastic component, with a density of 1.693, forms a very narrow but conspicuous band somewhat higher in the density gradient. As shown in Fig. 17 B, both components are sensitive to DNase digestion: 1-hr incubation,

TABLE II  
*Inhibitory Action of Hemin Deficiency upon the Effects of Acriflavine*

Sample	Aspect of the kinetoplast		
	Normal	Reduced	Not visible
A	2.0	52.5	45.5
B	10.7	84.2	5.1

Identical inocula of *Crithidia luciliae* were introduced in complete Boné's medium (culture A) and in the same medium lacking hemin (culture B). Both cultures received 20  $\mu\text{g}/\text{ml}$  acriflavine and were incubated for 24 hr. Growth was much slower in culture B. Smears of each culture were examined and the results are expressed in the same manner as in Table I.

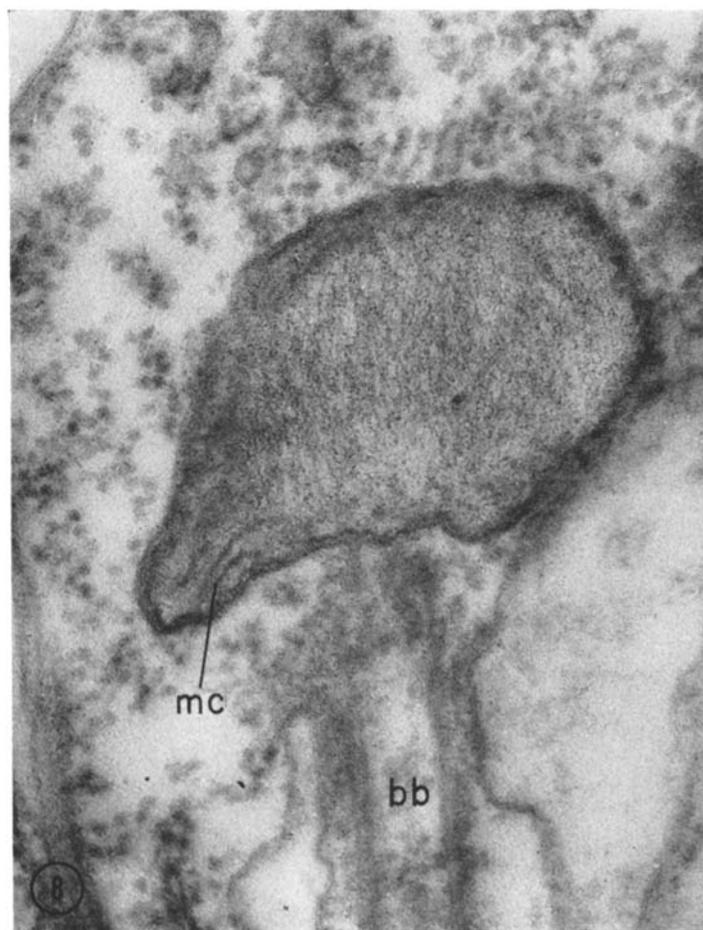


FIGURE 8 *Crithidia luciliae*. Kinetoplast of a normal cell. The whole contents of the organelle appear to be fibrils, about 30 Å thick. A few mitochondrial cristae (*mc*) indicate the site where the kinetoplast merges into the typical mitochondrial part of the chondriome. *bb*, basal body.  $\times 96,000$ .

at room temperature, with 0.2 mg/ml DNase (Worthington Corporation, Harrison, N.J.) crystallized once. The lesser constituent is almost totally absent from extracts of *T. mega* grown for 5 days in the presence of 2  $\mu\text{g}/\text{ml}$  acriflavine (Fig. 18). A stained smear of this culture shows that most cells are dyskinetoplastic and that the kinetoplast of the few individuals which still retain the organelle is much reduced in size.

#### DISCUSSION

Since Werbitzki's (41) first observation that the kinetoplast of infecting trypanosomes may apparently be lost after the injection of certain polycyclic organic chemicals into the infected host,

experimental induction of kinetoplastless trypanosomes has been repeatedly studied; a rather extensive review of the subject may be found in Mühlplfordt's papers (18–20). The most unexpected advance in this field is the rather recent discovery that the kinetoplast is still present in these abnormal hemoflagellates, but only as a rather empty double envelope. The inner fibrillar material, which stains with the Feulgen or Giemsa methods, is more or less completely lost (19, 37). The opinion was put forward, independently and almost simultaneously by Mühlplfordt (19) and by Trager and Rudzinska (37), that appropriate concentrations of acriflavine specifically affect the structure and amount of this fibrillar DNA-con-

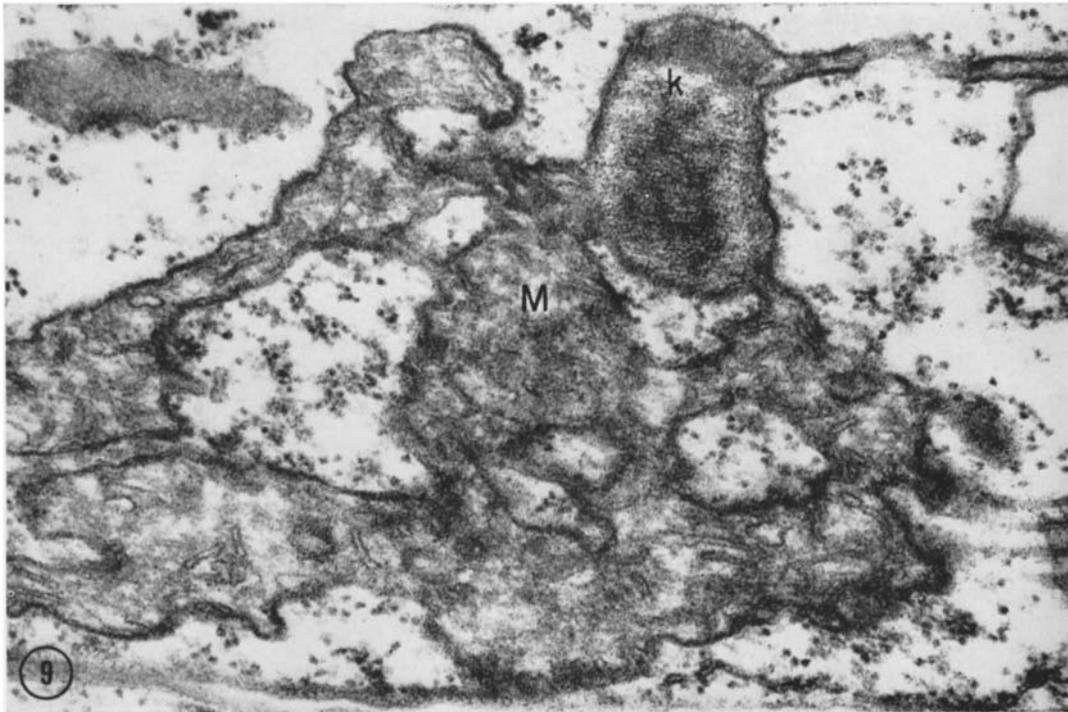


FIGURE 9 *Crithidia luciliae*. Detail of a normal cell. The section, which is longitudinal and close to the cell membrane, shows a part of the chondriome with its mitochondrial part (*M*) and its kinetoplast (*K*).  $\times 46,000$ .

taining material. Our own observations by light and electron microscopy, are in keeping with this opinion. It is difficult to ascertain, from morphological data, whether this loss is complete. However, the very unequal divisions of the kinetoplast which already appear in the second generation of crithidia after the addition of acriflavine, probably lead to cells completely devoid of kinetoplasmic DNA. Loss of kinetoplasmic DNA is also confirmed by our density gradient analyses which show the almost complete disappearance of the satellite DNA band in extracts of acriflavine-treated trypanosomes. In addition, our experiments with labeled thymidine now bring direct evidence for a specific inhibition of kinetoplasmic DNA synthesis.

A correlation between the suppressive activity of acriflavine on the kinetoplast and cell division has already been observed by Mühlpfordt (18), and our results with stationary-phase or hemin-deficient cultures now show quite definitely that the induction of dyskinetoplasty depends on cell di-

vision. Growth and the process of cell division itself are not directly affected by acriflavine, at the optimal concentration for producing dyskinetoplasmic cells, as was shown by Trager and Rudzinska for *Leishmania tarentolae* (37), and confirmed here with *C. luciliae*. Division of the organelle without compensating DNA synthesis can thus account for the stepwise decrease in the visible size of the kinetoplast. This idea is supported by the observation of Guttman and Eisenman (10) and by our observation that the kinetoplast is retained by hemoflagellates treated with concentrations of the dye that are high enough to bring multiplication to a stop.

The fact that the mode of interaction of acridine dyes with DNA is partially understood (15, 16) renders its adverse effect on kinetoplasmic DNA replication particularly interesting. Acridines are also known to affect extrachromosomal genetic materials, such as bacterial episomes, selectively (12, 13), and to induce respiratory deficiencies in yeasts (28). The mechanism of this specific dele-

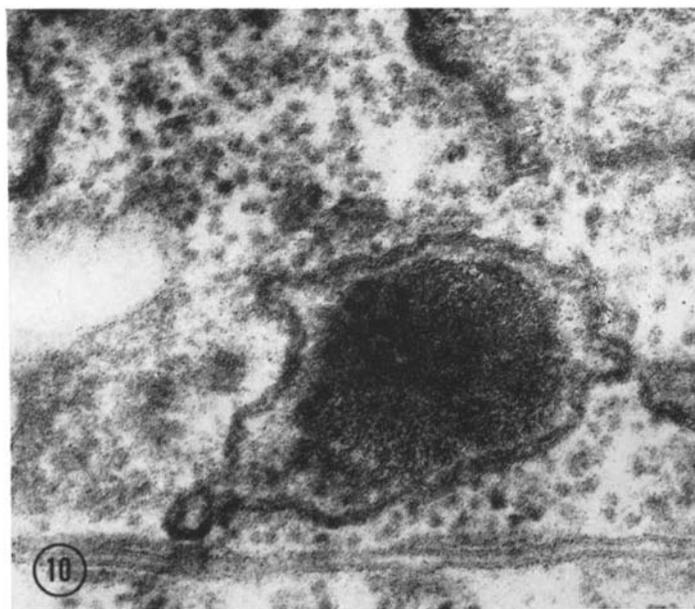


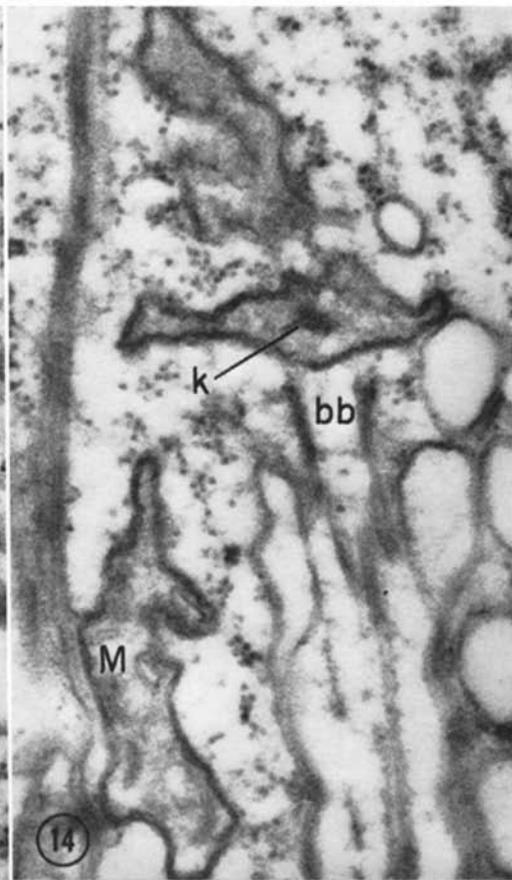
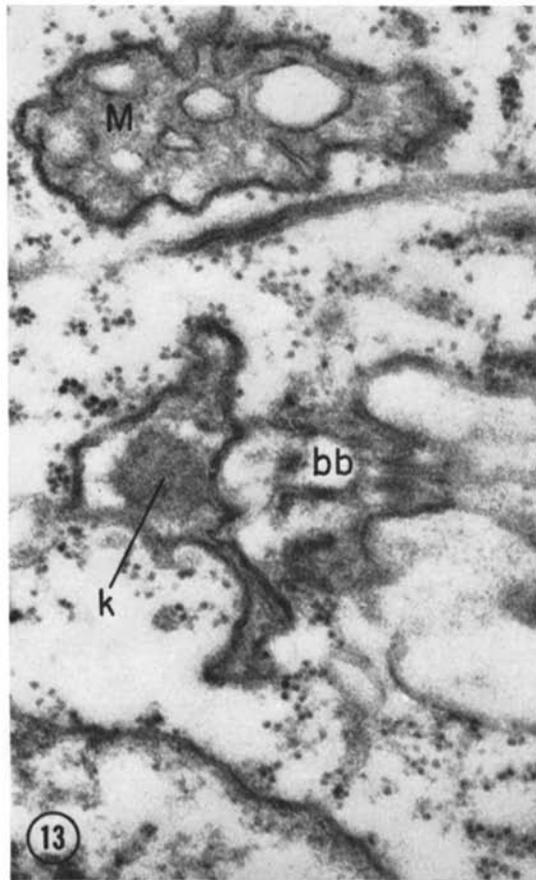
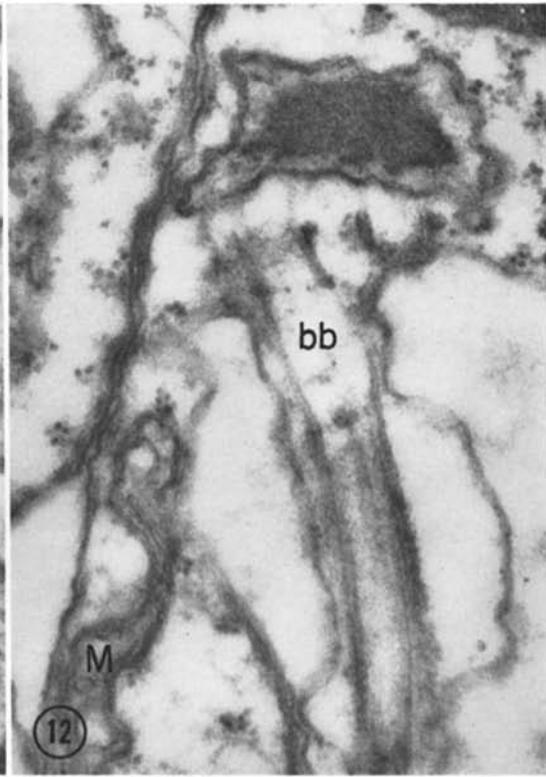
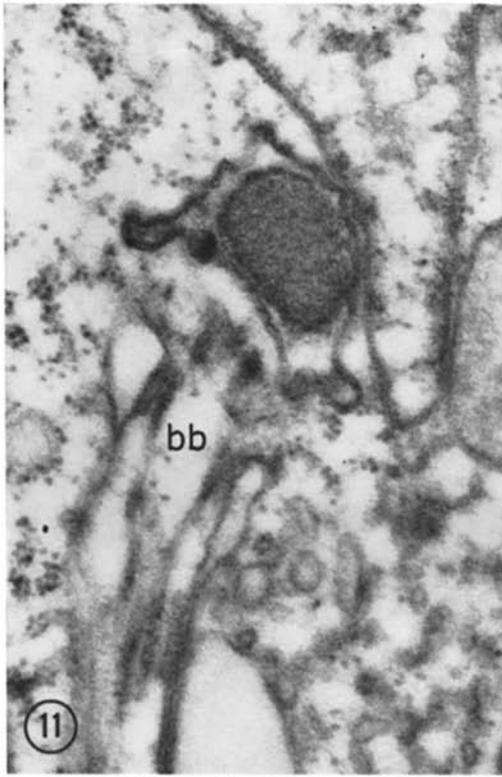
FIGURE 10 *Crithidia luciliae*, treated 3 days with acriflavine. The kinetoplast shown here is about half the normal size. Note the much denser packing of the inner fibrillar material and its segregation from the double membrane of the chondriome.  $\times 96,000$ .

tion or alteration of extrachromosomal genetic material is, nevertheless, far from clear.

It has been established that the binding of acridines by DNA involves the intercalation of dye molecules between specific base pairs and distortion of the DNA molecule (15, 16). At least two possible types of interference with mitochondrial DNA, leading to inheritable defects, might be thought to occur as a result of acridine binding: (a) direct inhibition of the replication process as a result of strand distortion, or (b) incorrect copy and synthesis of faulty DNA strands. This second possibility could explain mitochondrial deficiencies, but not complete loss of mitochondrial DNA, unless the error adversely affects the information corresponding to a specific factor of mitochondrial DNA synthesis. The extreme rapidity with which acriflavine shows its inhibitory action on thymidine- $^3\text{H}$  incorporation into the kinetoplast of *C. luciliae* clearly indicates that, in these cells, the replicative process is *directly* affected, according to the first hypothesis. However, it has been shown here that thymidine- $^3\text{H}$  incorporation into the kinetoplast is not entirely blocked by acriflavine, but that a residual incorporation of about 13% of the normal incorporation is maintained. This re-

sult is to be expected in a system in which the synthetic process can still be initiated but is afterwards prevented by some structural defect of the template, so that only short initial segments of the DNA would be copied.

The inhibitory action of acriflavine on kinetoplastic DNA synthesis and its suppressive effect on the organelle appear to be highly selective, since the nucleus remains apparently unaffected; an 87% decrease of thymidine incorporation in the kinetoplast can be observed although no inhibition whatsoever is found in the nucleus. A reason for this specific action might possibly be found in the higher A-T content of kinetoplastic DNA, compared to nuclear DNA (10): the capacity of DNA for acriflavine binding seems to depend on its base composition (38). Although eukaryotic and prokaryotic systems appear to be very different in many respects, it must be observed, nevertheless, that differences in base composition cannot account for the selective action of acriflavine on the F episome which, as shown by Marmur et al. (17), has the same buoyant density as *Escherichia coli* chromosome DNA. Other explanations could be related to the fact that kinetoplast DNA is not bound to histones (33), and might perhaps be



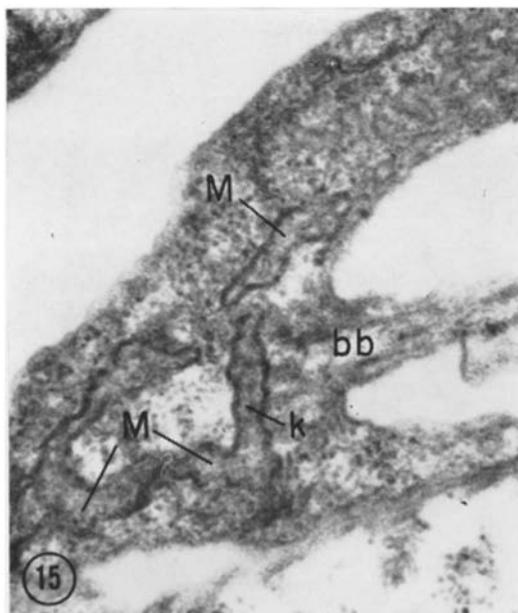


FIGURE 15 *Crithidia luciliae*. 3 days' treatment with acriflavine. The cell is dyskinetoplasic. The normal site of the kinetoplast (*k*), identified by means of the nearby basal body (*bb*), is occupied by a part of the chondriome which is now indistinguishable from the mitochondrion (*M*) which is reduced to small tubular profiles.  $\times 46,000$ .

more readily subject to acridine intercalation. The possibility must also be considered that the selective effect of acriflavine on the kinetoplast could be due to differences in the permeability characteristics of the membranes of the nuclear and the kinetoplasic systems. In this respect, it is worth mentioning that this differential permeability does not necessarily concern acriflavine:  $Mg^{++}$  concentration is known to affect acridine binding to DNA (21), and a higher capacity to accumulate  $Mg$  ions in the nucleus might be a sufficient condition for higher resistance of this organelle to acriflavine. In the case of *E. coli* and the F episome there is yet another hypothesis, the one proposed by

Jacob et al. (12), which deals with the differential sensitivity of the replication enzymes. This hypothesis should be kept in mind, since it is apparently equally satisfactory for the trypanosomal and bacterial systems.

The similarity between the kinetoplast and a bacterial nucleus has been mentioned already (25); this resemblance is striking in our material which has been fixed according to Ryter and Kellenberger. It should also be emphasized that the kinetoplast is best preserved by a fixative which is optimal for the conservation of the bacterial nucleus.

The ultrastructure of the other parts of the

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FIGURES 11-14 *Crithidia luciliae*, after 24 hr treatment with acriflavine. Different degrees in the reduction of the kinetoplast are shown: a general feature of these abnormal kinetoplasts is the condensed state of the DNA plasm and its detachment from the membrane. Note also the decrease in size of the whole organelle, as defined by its double membrane, and, in Figs. 12 and 13, the abnormal, vesicle-like aspect of some cristae in the mitochondrion (*M*). *K*, kinetoplast; *bb*, basal body.  $\times 46,000$ .

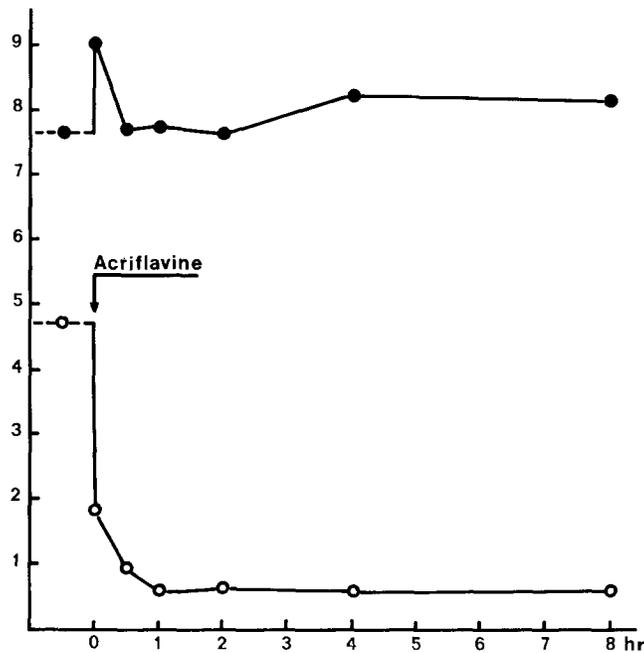


FIGURE 16 *Crithidia luciliae*. Effects of acriflavine (40  $\mu\text{g}/\text{ml}$ ) upon the incorporation of thymidine- $^3\text{H}$  in nuclear and kinetoplasmic DNA, as studied by radioautography. The mean number of silver grains counted over the nucleus (—●—) and the kinetoplast (—○—) of labeled individuals is plotted against the time, in hours, beginning with the addition of the dye to the culture. Labeling of the kinetoplast is rapidly and specifically inhibited. The small increase in nuclear labeling at 0 time is not considered significant.

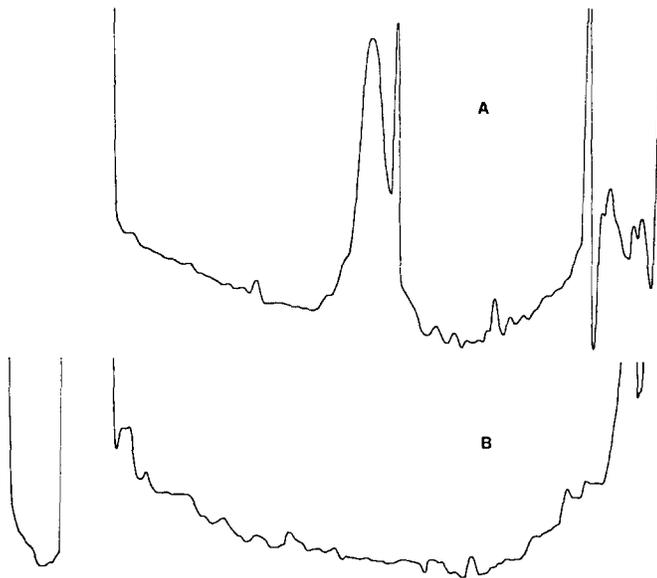


FIGURE 17 *A* CsCl density gradient analysis of total *Trypanosoma mega* DNA. The sharp peak at the right is attributed to kinetoplasmic DNA. *B* same extract as in *A*, but after DNase digestion.

mitochondrion is not severely affected by acridine treatment. The vesiculation of the mitochondrial cristae in acriflavine-treated *C. luciliae* has also been observed by Trager and Rudzinska in *Leishmania* (37). A significant observation, to be published elsewhere, is that oxygen consumption is

greatly diminished in *C. luciliae* that is deprived of kinetoplast or has a kinetoplast reduced in size. Similar observations, made by one of us (M.S.) several years ago on the culture form of *T. mega* (unpublished), suggest again that the kinetoplast must be considered as a peculiar mitochondrial

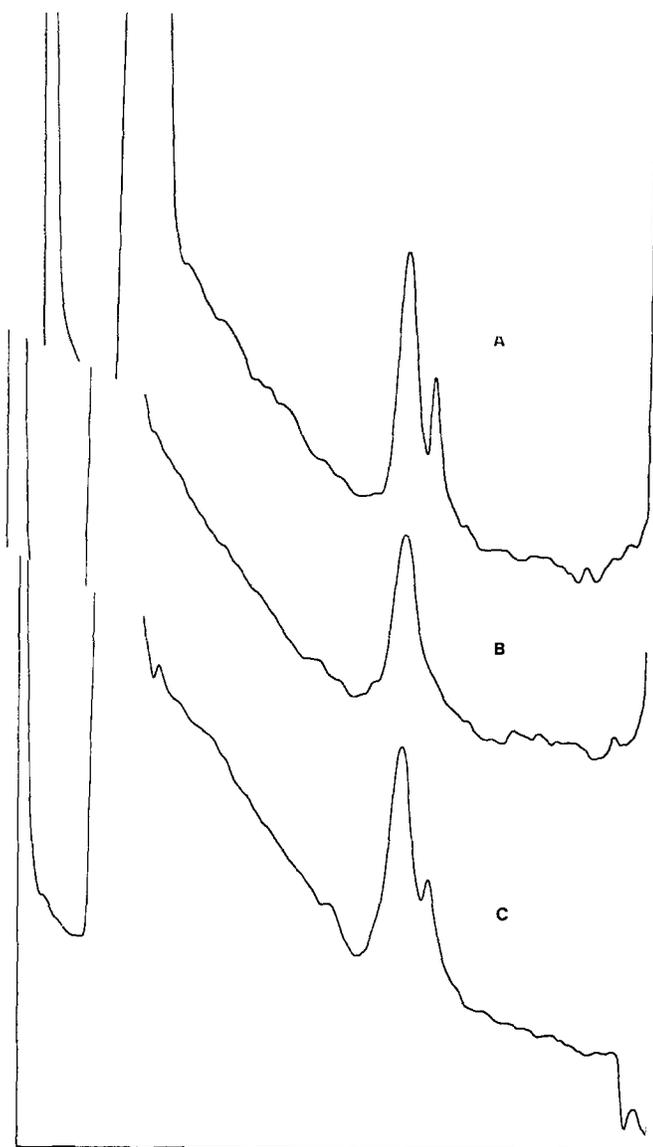


FIGURE 18 CsCl density gradient analyses of total DNA extracts of normal *T. mega* (A); of acriflavine-treated *T. mega* (B); and of a mixture of the two preceding extracts (C). The lighter DNA component (kinetoplasmic, presumably) is not present in diagram B.

genome, which, like the extrachromosomal genetic factor of yeast involved in respiration deficiency, appears to be exceptionally sensitive to acriflavine.

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