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Morphometric Data on the Ultrastructure of the Pleomorphic Bloodforms of *Trypanosoma brucei,* Plimmer and Bradford, 1899

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Introduction

Quite a number of electron microscopical studies of the trypanosomal cell dealing with the morphology and the functional aspects have been published in the last decade. VICKERMAN (1962, 1965, 1969) studied some aspects of the life-cycle in bloodstream forms, culture and vector forms. Special attention was thereby given to the alterations of the chondriome and the acquisition and loss of the surface coat. The same author demonstrated, combining ultrastructural with cytochemical light microscopic findings, that alterations in shape and size of the mitochondrial complex are associated with differences in enzymatic activities. Following previous light microscopical work on the "volutin" granules (ORMEROD, 1958, 1961, 1962, 1966; MICHEL, 1964, 1966), some cytoplasmic organelles and inclusions were studied by BIRD et al. (1966, 1970), MOLLOY & ORMEROD (1971). A few quantitative approaches to the complex morphological situation in trypanosomatids have recently been made (HUISENGA, 1970; MUSE et al., 1970; BROWN et al., 1972). Stereological principles, however, have never been applied in this context.

We therefore started to analyse morphometrically the different stages of *T. brucei,* the causative agent of the Nagana disease, during its life-cycle. We used the methods extensively described and adapted to biology by WEIBEL (1963, 1969), WEIBEL et al. (1966). In the present paper we deal with the quantitative differences in organelle content between pleomorphic bloodforms. We intend to correlate and discuss the quantitative findings with morphological and biochemical data obtained by other authors.

Material and Methods

a. Trypanosomes

The highly pleomorphic strain of *T. brucei* used for our study was STIB 33, originally derived from LAB 110 (EATRO) and cyclically passaged through *Glossina.* The strain was then kept by 34 syringe-passages in mice and stored deepfrozen in dry ice according to the method described by CUNNINGHAM et al. (1963). Seven series of white Ivanovas RA 25 male rats (150–200 gm) were used for our experiments, each series consisting of 5–10 animals. Each rat was inoculated i.p. with 0.15 to 0.4 cc of infectious blood diluted with saline to

10^{-2}–10^{-6}. The dosis and the concentration were varied in order to get different increases of parasitaemia from series to series. The parasitaemia was checked daily by tail blood examination. When heavily infected the rats were bled by heart-puncture around the time point of the first peak of parasitaemia (day 4–11). The blood samples from the infected animals could not be drawn at random time points because the transformation to stumpy forms, once started, proceeds very rapidly, so that it is difficult to get percentages of slender forms between 30 and 90 %. The time points for collection of the blood of the series 4 and 5 had therefore to be carefully chosen. The blood of each series of rats was pooled, mixed 1 : 1 under vigorous stirring with a tris-buffered-saline-glucose system (LANHAM & GODFREY, 1970) modified by ZAHALSKY (personal communciation): tris 5 gm, glucose 2 gm, NaCl 4 gm, $CaCl_2 \cdot 2H_2O$ 0.2 gm, Na_2EDTA 5 gm ad 1,000 ml H_2O; the pH was lowered dropwise with conc. HCl to 8.0.

Five thin smears per pool were made, fixed in methanol and stained in Romanowsky-Giemsa (Siegfried). These slides were used to determine the percentage of slender, intermediate and stumpy bloodforms of *T. brucei* (fig. 6).

Per slide examined we counted 150 up to 300 trypanosomes depending on the flagellates' density. The mean values of the five slides were calculated (table 1). This laborious procedure was necessary because slender, intermediate and stumpy forms cannot be identified with certainty in random transsections on electron micrographs.

Because of their biochemical similarities (VICKERMAN, 1965) the values for stumpy and intermediate forms were combined and compared to the ones of slender forms. For all these reasons the results obtained will be expressed as functions of the percentage of slender forms.

The trypanosomes of each blood pool were crudely separated from the erythrocytes, by centrifugation at 1,500 r.p.m. ($400 \times g$) and then

Table 1. Mean percentages and standard errors of slender, intermediate and stumpy forms for 7 series of *T. brucei* (STIB 33)

Series No.	Slender	Intermediate	Stumpy
1	13.0 ± 0.9	12.0 ± 0.7	75.0 ± 1.4
2	23.6 ± 1.4	15.6 ± 1.1	60.8 ± 1.7
3	24.5 ± 2.0	16.7 ± 0.9	58.8 ± 3.9
4	48.4 ± 1.1	27.9 ± 3.0	23.7 ± 3.4
5	88.8 ± 0.9	11.2 ± 0.9	–
6	98.3 ± 0.5	1.5 ± 0.5	0.2 ± 0.05
7	100.0 ± 0.0	–	–

by the isolation method according to LANHAM & GODFREY (1970) on a DEAE-cellulose column. An analogously modified tris-buffered-saline-glucose-system was used to wash out the trypanosomes (ZAHALSKY, personal communication: tris 5 gm, glucose 2 gm, NaCl 4 gm, $CaCl_2 \cdot 2H_2O$ 0.2 gm, KCl 0.2 gm, $MgCl_2 \cdot 6H_2O$ 0.2 gm ad 1,000 ml H_2O; the pH was adjusted dropwise with conc. HCl to 8.0).

b. Electron microscopical preparation and sampling

The separated trypanosomes were spun down at 3,000 r.p.m. (1,700 × g) and then fixed in 0.1 M cacodylate buffered (pH = 7.3) 2.5 % glutaraldehyde for 1½ hours at 4 °C. They were then briefly washed in cold 0.2 M cacodylate buffer with 5 % sucrose and encapsulated in a drop of warm (55 °C) buffered agar 2 % (Difco Bacto). Before the agar was solidified, the trypanosomes were centrifuged down at 4,000 r.p.m. (3,000 × g) to obtain the best possible density of the protozoans at the bottom of the agar drop. Small cubes of the agar containing the flagellates were then cut with a razor blade and further processed as small tissue samples. They were left overnight in two changes of buffer at 4 °C. Post-fixation was performed in 0.2 M cacodylate buffered 2 % osmium tetroxide for two hours at 4 °C. The samples were prestained in 70 % acetone containing 1 % uranyl-acetate, dehydrated in acetone and propylene-oxide and embedded in epon.

It can be assumed that by the former procedures the trypanosomes have randomly been distributed and oriented in the blocks. About 20 blocks per series were made. 5 blocks of each series were chosen at random. Each block was sectioned in one section-plane with diamond knives on a LKB Ultrotome III to obtain sections of the same thickness at an interference colour between grey and silver (600–800 Å). The sections were mounted on parlodion-carbon-coated 150 mesh copper grids, and post-stained in lead-citrate (REYNOLDS, 1963).

Finally, we got 5 grids per series. From each grid (= one block) the technically best section was selected for taking micrographs in a Philips EM 300. Per section, depending on the density of trypanosomes in the preparation, 20–30 exposures were taken in a systematic random sampling at a primary magnification of 7,400 × on a 70-mm roll film. The magnification of the microcsope was calibrated with a carbon grating replica (2,160 lines/mm – Nr. 1002, Fullam, Schenectady, N.Y.). Thus about 100–150 negatives were obtained per series. These were contact-printed on 70-mm roll film.

c. Morphometrical evaluation

The positive films were projected on the screen of a table projector (WEIBEL, 1969) and analysed at a final magnification of 33,000 times. The screen contained a test grid consisting of a square coherent double-

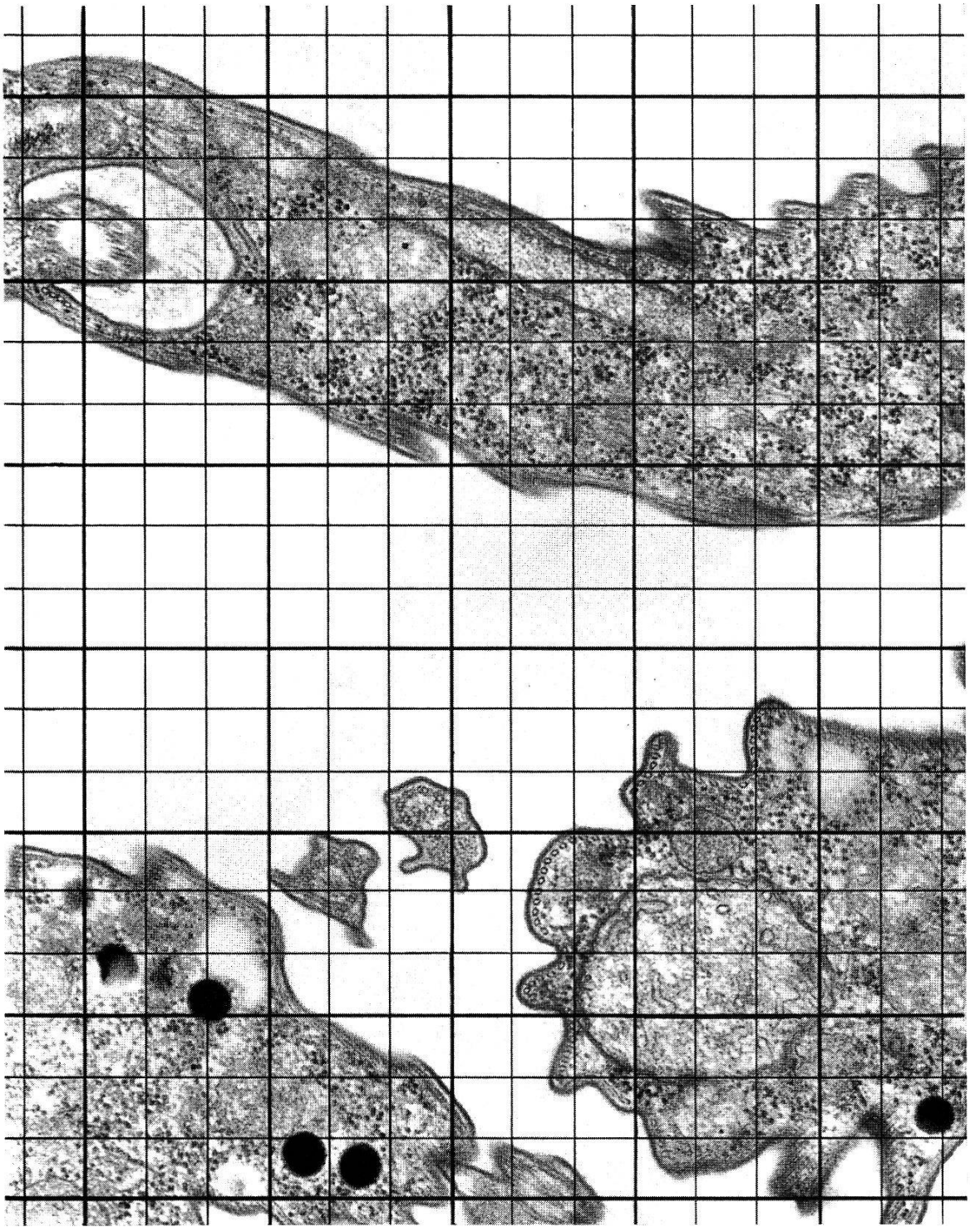

Fig. 1. Electron micrograph of randomly sectioned pleomorphic bloodforms of *T. brucei*. The picture shows part of the superimposed test point lattice for morphometric evaluation; the magnification of 33,000 times corresponds to that on the projection screen.

lattice test system with a lattice point ratio of 1 to 9. The distance between coarse points was 21 mm equivalent to 0.64 μ (fig. 1). Estimation of volume ratios and volume densities (V_V) was done by point counting. With the coarse test lattice the volume densities of the nucleus and of the cytoplasm of the cell body (flagellum excluded) were

evaluated. The fine point system was used to analyse the relative volumes of flagellum, reservoir (flagellar pocket), chondriome, vesicles and lipid inclusions.

The vesicles represent "type I granules" (BIRD et al., 1966, 1970), also called "peroxisome-like organelles" (VICKERMAN, 1969), whereas the lipid inclusions correspond to the "type II granules" (BIRD et al., 1966, 1970).

Per series 4 samples (80–100 micrographs) were evaluated. Each sample represented the area covered by 500 coarse points falling on nuclei and cytoplasm; this corresponded to an examined trypanosomal area of about 200 μ^2 per sample.

d. Calculations and statistical analysis

The results have been expressed in two forms. First, volume ratios and volume densities were calculated with respect to the total cytoplasmic volume (flagellum included). This is represented by P_{cy}; it was obtained by adding the fine points falling onto flagellum (P_{fl}) to the coarse points of the cytoplasm of the cell body (P_{cy}*) using the formula:

$$P_{cy} = 9 \cdot P_{cy}{}^{*} + P_{fl}$$

The following parameters were then calculated: nuclear-cytoplasmic ratio (nu/cy), reservoir to cytoplasm ratio (re/cy) and volume densities for flagellum (V_{Vfl}), mitochondrium (V_{Vmi}), vesicles (V_{Vve}), and lipid inclusions (V_{Vli}).

Secondly, the same parameters were expressed with respect to the cytoplasm of the cell body (without flagellum): the ratios nu/cy*, re/cy*, fl/cy* (flagellar-cytoplasmic ratio), and the volume densities V_{Vmi}*, V_{Vve}*, and V_{Vli}*.

As the measurements are not done on infinitely thin sections the volume densities obtained are subject to an overestimation due to the projection of the organelle segments on the image plane (Holmes' effect). As we were especially interested in a comparison of the pleomorphic forms a correction was omitted.

The regressions between volume densities and percentage of slender forms as well as the correlation coefficients (r) were calculated for all parameters.

An attempt was made to calculate absolute cell volumes of slender and stumpy forms. For this purpose the nuclear volume (V_{nu}) was estimated from camera lucida drawings of nuclear projections in Giemsa stained thin smears (fig. 6). Assuming the nuclei to be oblong rotation ellipsoids the long and short half-axis (a and b) of 50 nuclei per pleomorphic form have been measured. The absolute nuclear volume was then obtained with the formula:

$$V_{nu} = {}^4/_3 \pi \cdot a \cdot b^2.$$

From the nu/cy ratios and V_{nu} the absolute cytoplasmic volumes (V_{cy}) for the slender and the stumpy forms could be derived:

$$V_{cy} = \frac{V_{nu}}{nu/cy}$$

Results

a. General fine structure

The general ultrastructure of the bloodforms of *T. brucei* was described by VICKERMAN (1962, 1965, 1969). It can be summarized as follows: the slender form possesses a free flagellum, a reservoir (= flagellar pocket) and a slightly elongated nucleus in the middle part of the cell (fig. 2). The mitochondrium, which consists of but one practically cristaeless tube extending along the cell, originates in the subterminal kinetoplast. Additionally, one recognizes a Golgi complex lying near the reservoir, profiles of rough surfaced and smooth surfaced endoplasmic reticulum, as well as free ribosomes. Typically, membrane-bounded vesicles measuring 0.25–0.35 μ in diameter and showing a floccular content can also be observed (type I granules = volutin granules = lysosomes, BIRD et al., 1966, 1970; MOLLOY & ORMEROD, 1971). VICKERMAN (1969) called these vesicles "peroxisome-like" organelles and presumed that these structures represent the site of extra-mitochondrial terminal respiration. Moreover, darkly stained inclusions, never bounded by a unit membrane, most probably lipids, are scattered throughout the cytoplasm (type II granules = "endoplasmic reticular granules", BIRD et al., 1970; MOLLOY & ORMEROD, 1971). The whole cell is surrounded peripherally by regularly arranged microtubules (periplast-tubules), which run beneath the cell membrane. The latter is uniformly covered with a surface coat (VICKERMAN, 1969).

The stumpy form shows some structural differences: the free flagellum is very short or absent. The mitochondrium possesses more elaborate features; it is larger and its inner membrane yields many mitochondrial tubules. This transformation from the simple slender form to the stumpy form passes through intermediate stages.

b. Quantitative results (table 2)

1. Nuclear-cytoplasmic volume ratio (nu/cy)

In 6 series the nu/cy varies between 0.101 and 0.130, not showing any statistically significant differences. In one series (Nr. 5) the nu/cy shows a significant increase to 0.157 (fig. 3). This might be due to the

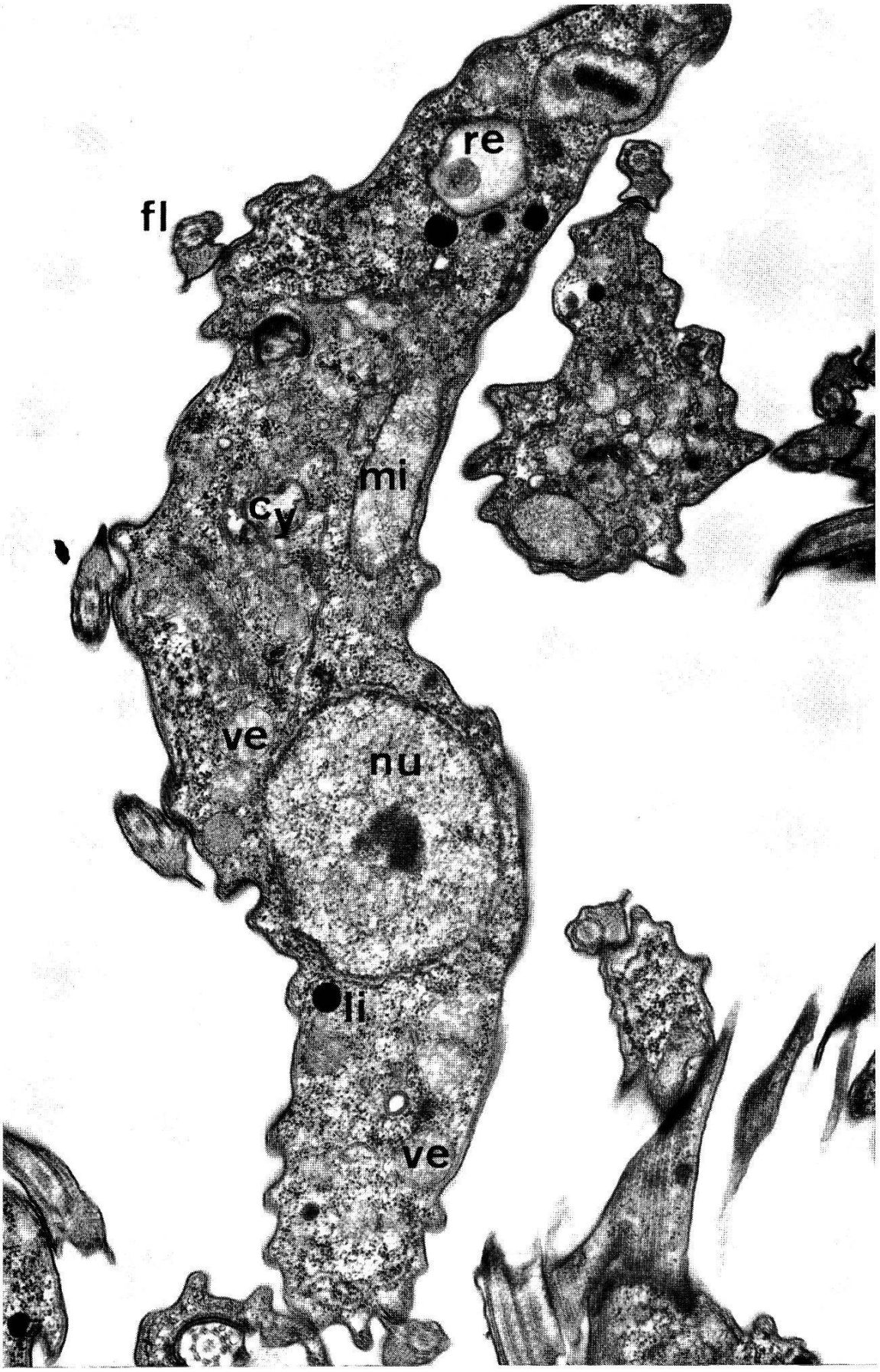

Fig. 2. General ultrastructure of *T. brucei*. nu = nucleus; cy = cytoplasm; fl = flagellum; re = reservoir (flagellar pocket); mi = mitochondrium; ve = vesicles; li = lipid inclusions. × 22,000.

Table 2. Means and standard errors of the parameters calculated with respect to the total volume of cytoplasm (flagellum included)

Series	1	2	3	4	5	6	7
nu/cy	0.130 ± 0.013	0.115 ± 0.008	0.101 ± 0.016	0.124 ± 0.009	0.157 ± 0.007	0.130 ± 0.008	0.109 ± 0.011
re/cy	0.031 ± 0.009	0.030 ± 0.002	0.020 ± 0.003	0.011 ± 0.003	0.013 ± 0.003	0.011 ± 0.0004	0.008 ± 0.001
V_{Vfl}	0.113 ± 0.005	0.127 ± 0.011	0.146 ± 0.004	0.157 ± 0.008	0.174 ± 0.004	0.178 ± 0.003	0.182 ± 0.014
V_{Vmi}	0.085 ± 0.008	0.074 ± 0.004	0.057 ± 0.005	0.048 ± 0.002	0.030 ± 0.002	0.020 ± 0.002	0.022 ± 0.002
V_{Vve}	0.035 ± 0.002	0.040 ± 0.003	0.036 ± 0.003	0.042 ± 0.002	0.044 ± 0.003	0.037 ± 0.002	0.032 ± 0.001
V_{Vli}	0.011 ± 0.001	0.008 ± 0.0008	0.009 ± 0.0004	0.009 ± 0.001	0.017 ± 0.002	0.008 ± 0.001	0.009 ± 0.0005

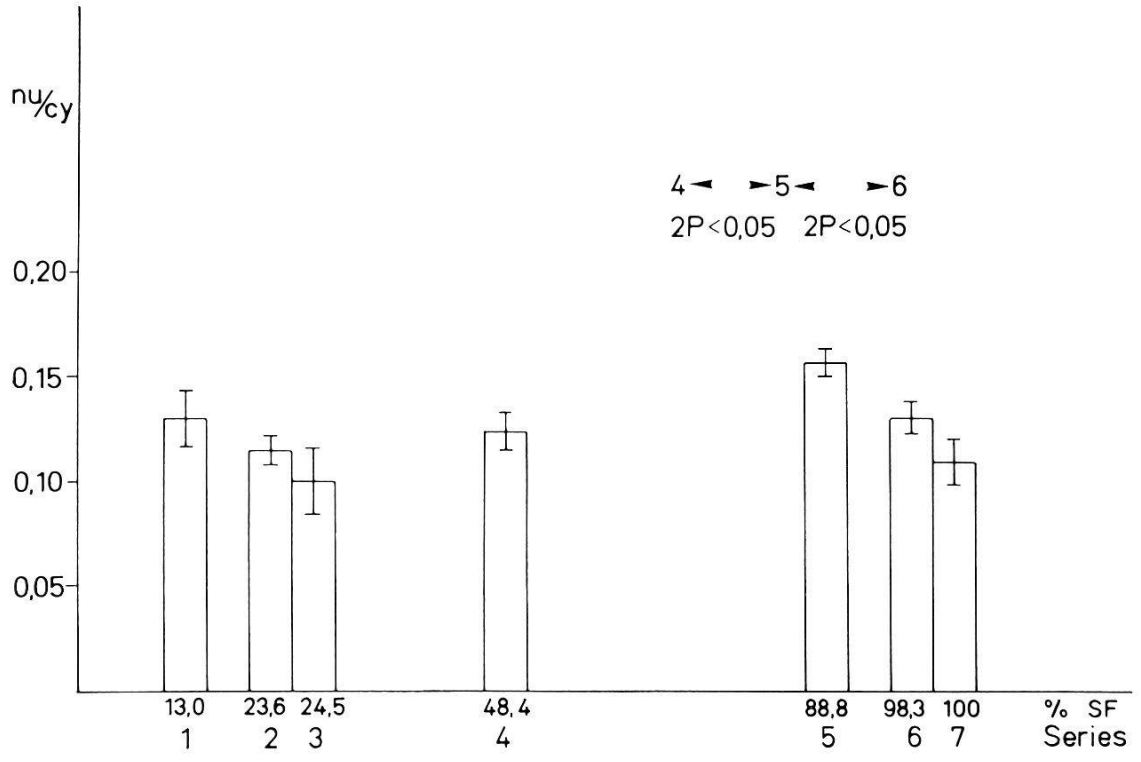

Fig. 3. Nuclear-cytoplasmic volume ratios of the 7 series, plotted against the percentage of slender forms (SF). Height of the columns represents means, brackets ± 1 standard error.

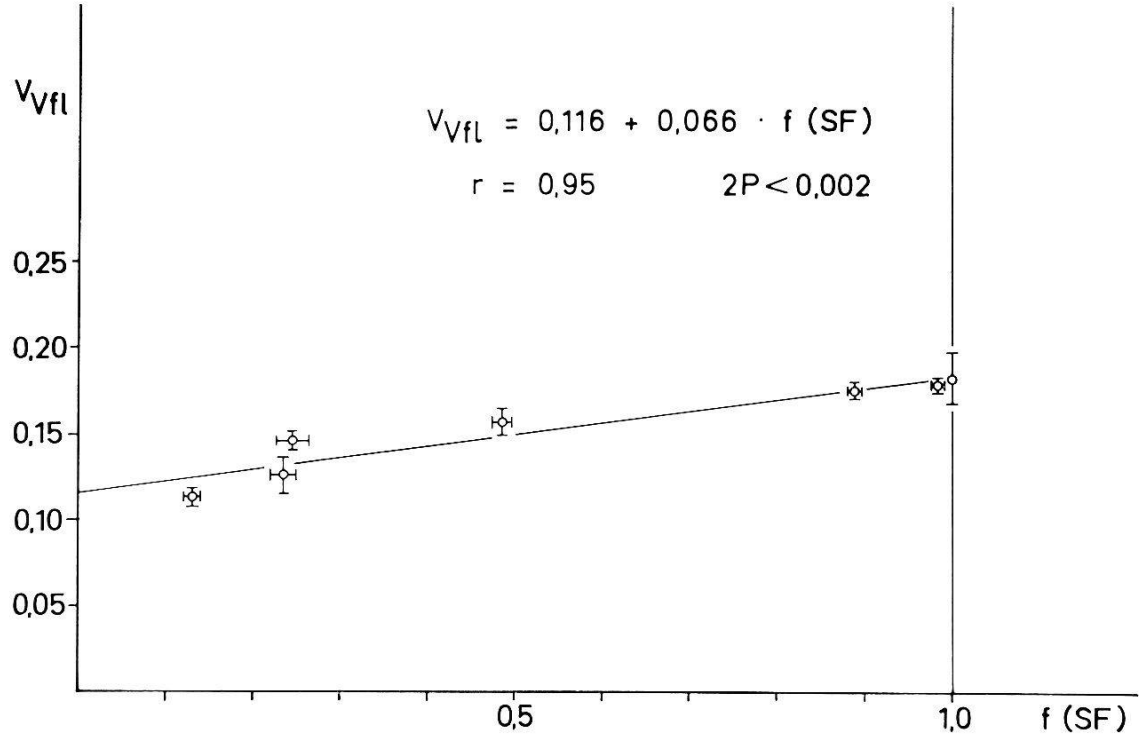

Fig. 4. Flagellar volume density (V_{Vfl}) plotted against frequency (f) of slender forms (SF). The straight line represents the regression line for V_{Vfl} drawn after the given formula. r is the correlation coefficient and 2P gives the significance level for r in the two-tailed test. Circles = means; brackets = ± 1 standard error.

high frequency of cell divisions observed at this moment and/or to the start of transformation into intermediate forms (table 1).

2. Reservoir to cytoplasm ratio (re/cy)

re/cy shows a definite decrease from 0.031 in series 1 down to 0.008 in series 7. However, nothing can be said about the linearity of the decrease, since we find a relatively low value (0.011) already in series 4.

3. Volume density of flagellum (V_{Vfl})

This parameter increases gradually from 0.113 in series 1 (13 % slender forms) up to 0.182 in series 7 (100 % slender forms). Fig. 4 shows that the increase is proportional to the percentage of slender forms and that the calculated regression line fits well to the points. The correlation coefficient reaches 0.95, its significance $2\,P < 0.002$.

4. Volume density of mitochondrium (V_{Vmi})

With increasing percentage of slender forms the V_{Vmi} decreases from 0.085 to 0.022. The data are represented in fig. 5; they fit closely to the calculated regression line, the correlation coefficient being −0.97. The significance level is $2\,P < 0.001$.

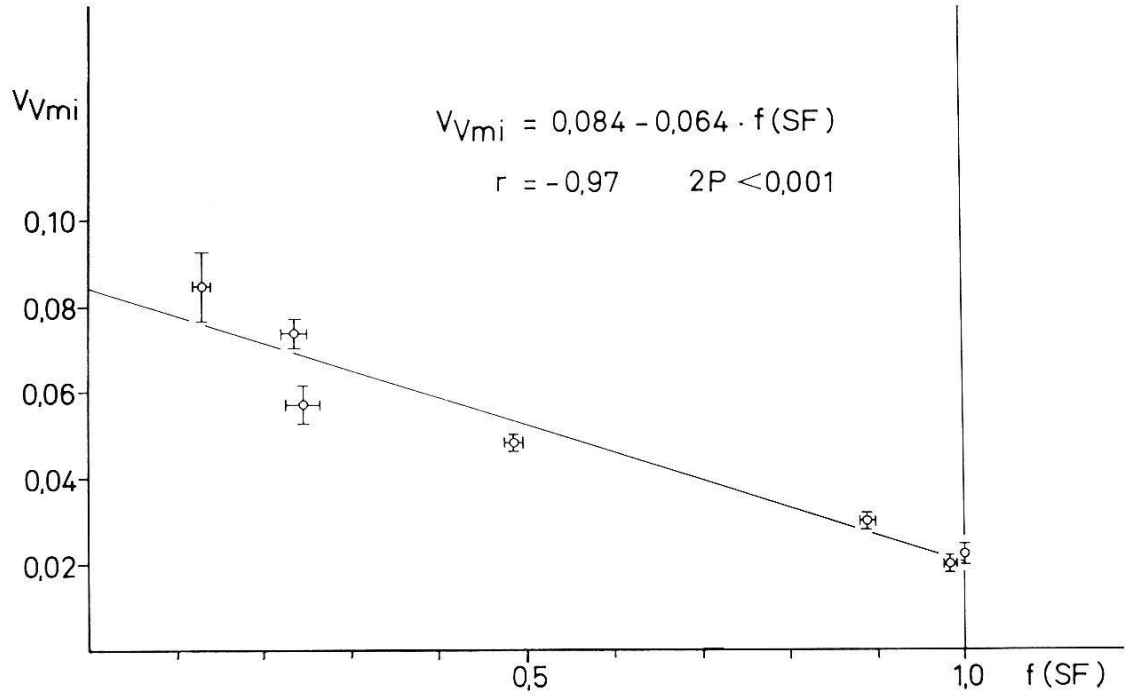

Fig. 5. Mitochondrial volume density (V_{Vmi}) plotted against frequency (f) of slender forms (SF). The calculated regression line for V_{Vmi} is drawn (see formula). r is the correlation coefficient and 2P gives the significance level for r in the two-tailed test. Circles = means; brackets = ± 1 standard error.

5. Volume densities of vesicles (V_{Vve}) and lipid inclusions (V_{Vli})

Both parameters do not show any clear trend depending on the amount of slender forms. V_{Vve} varies between 0.032 and 0.044. V_{Vli} ranges from 0.008 to 0.017. The high values in series 5 (0.017) is possibly due to a preparation artefact; for unknown reasons the lipid inclusions of this series appeared swollen.

Table 3 shows the corresponding results (paragraphs 1–5) calculated with respect to the cytoplasmic volume of the cell body (without flagellum). The results are naturally increased when compared to those presented in table 2. These values might be of interest for studies concerning the organelles of the cell body.

6. Cell volumes of slender and stumpy forms (flagellum included)

The absolute nuclear volumes for the slender and stumpy forms (fig. 6) derived from light microscopic measurements are 3.0 μ^3 and 7.4 μ^3, respectively.

A mean nu/cy was calculated from 6 series, the nu/cy-value of series 5 being excluded for reasons mentioned under section 1 of the quantitative results. The mean ratio thus being 0.118, the absolute cytoplasmic volumes are 25.4 μ^3 for the slender form and 62.7 μ^3 for the stumpy form. This corresponds to a total cell volume of 28.4 μ^3 and 70.1 μ^3, respectively (table 4).

Table 3. Means and standard errors of the parameters calculated with respect to the cytoplasmic volume of the cell body (without flagellum)

Series	1	2	3	4	5	6	7
nu/cy*	0.147 ± 0.015	0.131 ± 0.008	0.117 ± 0.018	0.148 ± 0.011	0.191 ± 0.009	0.158 ± 0.010	0.134 ± 0.015
re/cy*	0.035 ± 0.010	0.034 ± 0.002	0.023 ± 0.004	0.013 ± 0.003	0.016 ± 0.003	0.013 ± 0.0005	0.010 ± 0.001
fl/cy*	0.127 ± 0.007	0.145 ± 0.014	0.171 ± 0.006	0.187 ± 0.011	0.211 ± 0.006	0.216 ± 0.004	0.223 ± 0.021
V_{Vmi}*	0.095 ± 0.009	0.084 ± 0.004	0.067 ± 0.006	0.057 ± 0.002	0.036 ± 0.002	0.024 ± 0.001	0.027 ± 0.002
V_{Vve}*	0.039 ± 0.002	0.045 ± 0.003	0.043 ± 0.003	0.050 ± 0.002	0.053 ± 0.003	0.045 ± 0.003	0.039 ± 0.002
V_{Vli}*	0.012 ± 0.001	0.009 ± 0.001	0.010 ± 0.0005	0.011 ± 0.001	0.020 ± 0.003	0.009 ± 0.001	0.011 ± 0.001

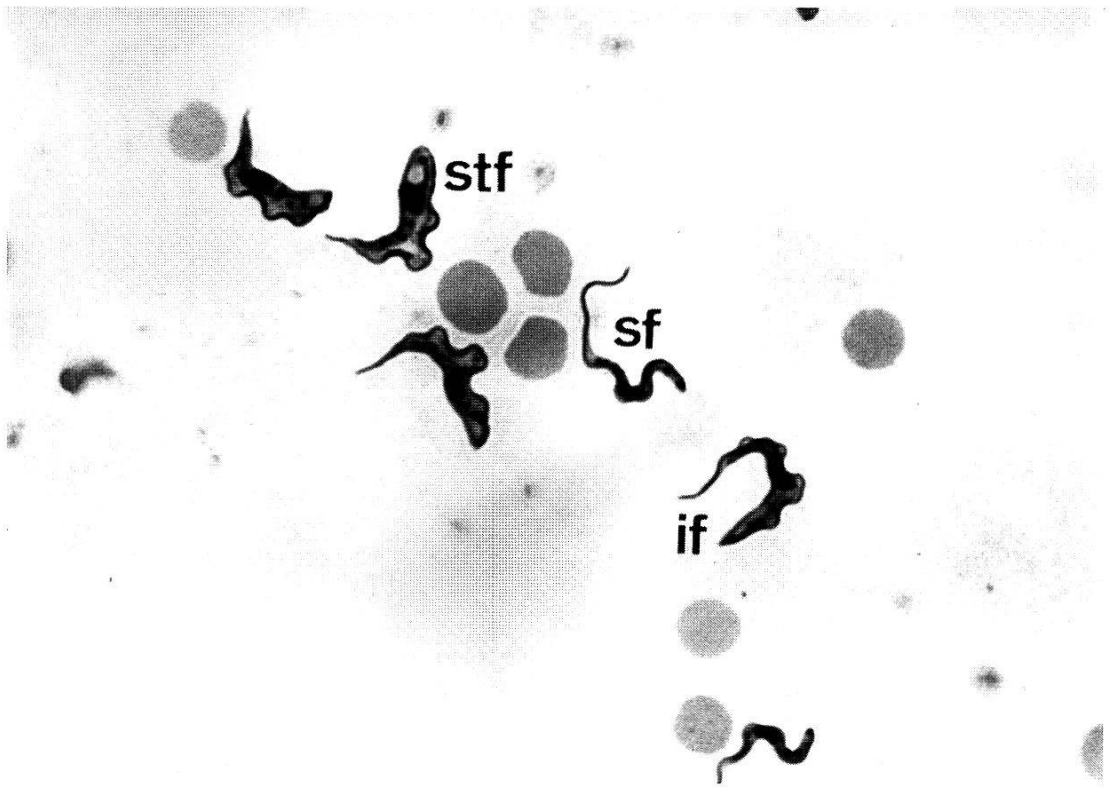

Fig. 6. Light micrograph of a Giemsa stained thin smear showing the pleomorphic bloodforms of *T. brucei* (sf = slender form; stf = stumpy form; if = intermediate form). × 1,875.

Table 4. Comparison of the parameters of a slender with an extrapolated stumpy form of *T. brucei*

Parameter	Slender form	Stumpy form
Total cell volume, μ^3	28.4	70.1
Absolute volume of cytoplasm (V_{cy}), μ^3	25.4	62.7
Absolute volume of flagellum (V_{fl}), μ^3	4.6	7.3
Absolute volume of nucleus (V_{nu}), μ^3	3.0	7.4
re/cy	0.008	0.030
V_{Vmi}	0.022	0.084
V_{Vfl}	0.182	0.116
V_{Vve}	0.032	0.038
V_{Vli}	0.009	0.009

Knowing the respective V_{Vfl}, the absolute volumes of the flagellum (V_{fl}) can easily be deduced from the absolute cytoplasmic volumes. The values are 4.6 μ^3 for slender and 7.3 μ^3 for stumpy forms.

Discussion

As far as we know, it is the first time that stereological methods have been applied to protozoans. The problems with which one is faced in the present case is that, on the one hand, it is easy to investigate blood samples containing 100% slender forms, but it is not possible to get a pure population of stumpy forms, and on the other hand, that it is impossible to identify the pleomorphic type of a randomly sectioned trypanosome in an electron micrograph. The relative composition of the cytoplasm of the stumpy forms can, however, be obtained by extrapolating the regression lines obtained from different series containing a known and variable proportion of slender forms. However, this estimation of a "calculated stumpy form" has to be considered with caution, since extrapolation is only permitted if the regression function is known. We have assumed linearity on the basis of the following argument. We have expressed our data in relation to the frequency of slender forms, f. The parameter, P, obtained at any point, fx, between $0 < fx < 1$ may be safely assumed to be the arithmetic mean of the parameters P1 and P2 of slender and stumpy forms, respectively, weighted according to their respective frequency:

$$P(fx) = fx \cdot P1 + (1-fx) \cdot P2.$$

This is evidently a linear function from which the parameter pertaining for stumpy forms is obtained by setting $fx = 0$.

The parameters of this "calculated stumpy form" are compared to those of the slender form in table 4.

As the general results indicate the nu/cy and the volume densities of vesicles and of lipid inclusions show no significant trend. There are, however, marked quantitative differences between the two forms in the volume densities of the chondriome, the flagellum and the reservoir.

The higher value for re/cy in the stumpy form could be ascribed to a higher pinocytotic activity, since it is known that the flagellar pocket is the site of pinocytosis in *T. brucei* (e.g. GEIGY et al., 1970).

The results concerning V_{Vfl} indicate a larger volume density of the flagellum in the slender form. Considering the fact, however, that absolute stumpy cell volume is about almost 3 times larger than cell volume of slender forms, the absolute flagellar volume is higher in stumpy forms.

VICKERMAN (1965) demonstrated that the mitochondrium of the slender form is not active and that respiratory enzymes, such as NAD-diaphorase, could only be traced in discrete extramitochondrial bodies. RYLEY (1964) and VICKERMAN (1965) have also given some evidence that a functional Krebs cycle is absent in slender forms, whereas it is partly acquired when the slender form transforms through intermediate stages into the stumpy form. This transformation is accompanied by changes in size and shape of the chondriome (VICKERMAN, 1962, 1965). The mitochondrium is then positive for NAD-diaphorase (VICKERMAN, 1965). All these observations are confirmed by our quantitative results, which show a significantly greater relative volume of the stumpy form's mitochondrium.

The terminal respiration in slender bloodforms of the *brucei*-subgroup trypanosomes is effected by a L-α-glycerophosphate oxidase system, which is supposed to be located in extramitochondrial bodies (see FULTON & SPOONER, 1959; GRANT & SARGENT, 1961; GRANT et al., 1961; RYLEY, 1964, and the review article by HILL & ANDERSON, 1970). These extramitochondrial bodies might be identical with the SH-granules seen in the light microscope by MICHEL (1966) and with the "peroxisome-like" organelles of VICKERMAN (1969). These organelles probably correspond to our vesicles (ve, fig. 2).

It was now interesting to know whether these organelles, possibly responsible for the para-mitochondrial terminal respiration, also show alterations in their relative volume densities concomitantly with the respiratory switch in the mitochondrium. This is not the case, the values for V_{Vve} being practically the same in all seven series (table 2). It has not been demonstrated yet that these extra-mitochondrial bodies lose their enzymatic activity during transformation into stumpy forms. It could well be that the vesicles persist, with or without loss of activity, in the course of the whole life-cycle to be used again when the trans-

formation of the metacyclic salivary gland form into the slender blood form takes place. The role of these organelles needs further clarification. For the moment, it seems important to note that peroxidase ("peroxisome-like organelles": VICKERMAN, 1969) but no phosphatase activity ("lysosomes": BIRD et al., 1970) could be demonstrated in this type of organelles in the bloodforms of *T. brucei* (STEIGER, in prep.).

In our observations the lipid inclusions show similar V_{Vli} for all series. Their lipid nature has been suggested by VENKATESAN (1972) and has been demonstrated in electron microscopy by lipid extraction (STEIGER, in prep.). These inclusions are classified as "type II granules" (e.g. BIRD et al., 1966). An association with the endoplasmic reticulum ("endoplasmic reticular granules": BIRD et al., 1970; MOLLOY & ORMEROD, 1971) could never be observed. Both our vesicles and the lipids were regarded by several authors as being "volutin" granules, however, without any clear definition. HERBERT (1965) working with *T. theileri* stated that inclusions defined as "volutin" granules might be made up by several types of granules and inclusions of different function. The observation by ORMEROD (1961) that in *T. rhodesiense* "volutin" granules occur in a higher number in stumpy forms than in slender forms is not directly comparable to our quantitative findings for *T. brucei,* since ORMEROD worked with the absolute granule numbers per trypanosome. In the present study, however, relative volumes are considered, which allow a more reasonable functional interpretation.

Based on our results we cannot confirm in any way the proposition of BIRD et al. (1970) that in *T. brucei* "Type II granules do not occur in the early "long-thin" form, but as trypanosomes age to become "short-stumpy", the number of Type I granules decrease and those of Type II increase." Strain-specificities may account for these differences.

The results concerning the absolute volumes for slender and stumpy forms are surprising. It was found that the stumpy form, very often described as being the small form, proved to be about 3 times larger in cell volume than the long slender form. It must be added that the values found are approximatious and are to be taken with caution, because they were obtained using two different techniques. The nu/cy was determined on material prepared for electron microscopy, whereas the nuclear volumes were measured on methanol fixed thin smears, where trypanosomes are known to be somewhat distorted. However, it can be assumed that this difference in size between stumpy and slender forms is relevant, since it is most improbable that the preparation techniques affect preferentially only one form.

The first morphometric data obtained on trypanosomes in a few experiments show that stereological methods can advantageously be applied to protozoans. With further quantitative studies on other trypanosome material (other strains, their bloodforms, vector and cul-

ture forms) we hope to contribute to a complete quantitative characterization of the life-cycle of *T. brucei*.

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Zusammenfassung

Die pleomorphen Blutformen von *Trypanosoma brucei* (STIB 33) wurden erstmals morphometrisch untersucht.

Die 7 bearbeiteten Proben (Serien) enthielten zwischen 13 % bis 100 % schlanke Formen (slender forms).

Von 5 Blöcken jeder Serie wurden bei geeigneter Vergrößerung 100 bis 150 Aufnahmen von Trypanosomenschnitten gemacht. Ihre quantitative Auswertung erfolgte mit einem Tischprojektor unter Anwendung des Punktzählverfahrens.

Für jede Serie wurden folgende Parameter erfasst: die Kern-Plasma-Relation, die Reservoir-Cytoplasma-Relation sowie die Volumendichten des Flagellums, der Mitochondrien, der Vesikel und der Lipide.

Durch Berechnen linearer Regressionen zwischen den ermittelten Parametern und dem Prozentanteil schlanker Formen konnten die quantitativen Daten der

stumpfen Formen (stumpy forms) abgeleitet werden. Dies ermöglichte einen quantitativen Vergleich der ultrastrukturellen Zusammensetzung der schlanken und stumpfen Formen und eine Analyse ihrer biochemischen Unterschiede.

Speziell konnten die Ergebnisse anderer Autoren unterstützt werden, wonach die Umwandlung der schlanken zu stumpfen Formen von einer erheblichen Vergrößerung und Aktivitätsänderung des Mitochondriums begleitet ist. Erstaunlicherweise blieben jedoch die Volumendichten der Vesikel und Lipide weitgehend gleich.

Zusätzlich wurden die absoluten Volumina der pleomorphen Blutformen berechnet. Es konnte gezeigt werden, daß das Volumen der stumpfen Form bedeutend größer ist als dasjenige der schlanken Form. Dies traf auch für die Volumina des Kerns und der Geißel zu.

Bei den erhaltenen Resultaten muß beachtet werden, daß diese eventuell vom verwendeten *T. brucei*-Stamm teilweise abhängig sind.

Résumé

Les formes pléomorphes de *Trypanosoma brucei* (STIB 33) dans le sang du rat ont été étudiées pour la première fois par des procédés morphométriques.

Les 7 séries obtenues comprenaient entre 13% et 100% de formes élancées (slender forms).

A partir de coupes minces de 5 blocks par série 100 à 150 micrographies électroniques ont été prises. Leur analyse quantitative a été réalisée à l'aide d'un projecteur de table dont l'écran contenait un double système de points équidistants.

Les relations volumétriques entre noyeau et cytoplasme et entre réservoir et cytoplasme ainsi que les densités de volume du flagelle, du chondriome, des vésicules et des lipides ont été etablies.

En calculant les régressions linéaires entre les paramètres cités et le pourcentage des formes élancées, les données quantitatives des formes courtes (stumpy forms) ont pu être extrapolées. Ceci a permis de dresser une comparaison quantitative de la composition ultrastructurale des formes longues et courtes, ainsi qu'une analyse de leurs différences biochimiques.

Les résultats d'autres auteurs, selon lesquels la transformation des formes élancées en formes courtes était accompagnée par un agrandissement du chondriome et d'un changement de son activité, ont pu être confirmés. Par contre, les volumes relatifs des vésicules et des lipides ne montraient pratiquement pas de changement.

Parallèlement, le volume absolu des formes pléomorphes a été calculé. Ceci a permis de démontrer que le volume cellulaire des formes courtes dépassait largement celui des formes longues. La même constatation a pu être faite pour le volume nucléaire et le volume de flagelle.

Les auteurs soulignent cependant que les résultats présentés peuvent en partie dépendre de la souche de *T. brucei* employée.