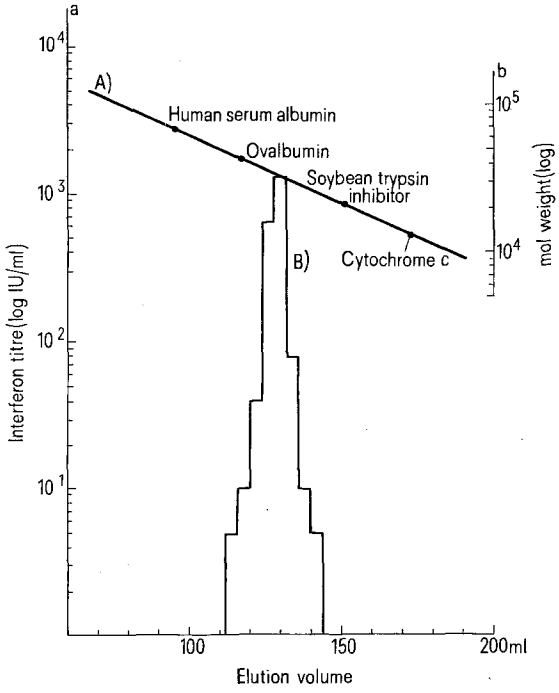
Optical density of the fractions was read spectrophotometrically (spectrophotometer SFD-2, USSR) at a wavelength of 280 nm; blue dextran was additionally read at 625 nm, and cytochrome c at 412 nm.

2-ml sample of the concentrated interferon preparation, containing blue dextran and cytochrome c was layered on top of the column. Interferon titre in interferon units (IU) per ml of the 4-ml effluent fractions was determined according to CPE inhibition method in tube monolayer TGK cell cultures, using vesicular stomatitis virus, strain Indiana, as challenge virus[1].



Chromatography of SFV-induced tortoise interferon on Sephadex G-100 column. A) Calibration curve with use of reference proteins (standard markers). B) Elution profile of 1 ml concentrated interferon preparation.

The results of the chromatography on Sephadex G-100 column of the interferon preparation, obtained from SFV infected TGK cells, are presented in the Figure. As can be seen, a single peak of interferon activity was established. A molecular weight of 33,500 $\pm$ 4.5 % of the virus-induced tortoise interferon was calculated by plotting the elution volume of the interferon peak on the calibration curve of the column.

Evidently, the determined molecular weight of the interferon of tortoise, class Reptilia, is within the 25,000–38,000 range found by other authors for virus-induced interferons in cell cultures of birds[4–6] and mammals[7–9].

By using isoelectric focusing in polyacrylamide gels for purification of mouse and human interferons, CARTER[10] obtained a dissociation of the native interferon molecule which he assumes to be a dimer of similar or identical subunits (with molecular weght of 19,000 or 12,000, respectively). Our study on the tortoise interferon by the same technique is in progress.

Zusammenfassung.. Das Molekulargewicht des Interferons, durch Selmiki Forest Virus in Zellkulturen von Schildkrötenniere induziert, wurde mittel Gelchromatographie mit Sephadex G-100 bestimmt und es ergab sich ein Wert von 33,500.

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Quantitative Ultrastructural Differences in the Mitochondrium of Pleomorphic Bloodforms of *Trypanosoma brucei*[1,2]

In a previous study[3] we investigated the quantitative alterations occuring in the organelle content of a pleomorphic strain of *Trypanosoma brucei* (STIB 33) during the transformation of the slender to the stumpy form. In this context, distinct differences in the volume density of the mitochondrium (V_{Vmi}) were shown to exist between slender and stumpy forms. The increased V_{Vmi} for the stumpy forms was found to correlate with morphological and biochemical observations on the mitochondrial changes in bloodstream trypanosomes[4,5].

The present paper describes our investigations of the changes in the surface densities of the inner (S_{Vmii}) and outer (S_{Vmio}) mitochondrial membranes.

Material and methods. The strain of *T. brucei* (STIB 33) and the preparation methods for electron microscopy have already been described[3]. The same 7 series with determined frequency of slender forms (f(SF), Table I) were used for the present study. In each series, 4 blocks were randomly chosen and thin sections cut in 1 section plane. From 1 section per block 40 micrographs were taken by systematic random sampling on 35 mm rollfilm at a primary magnification of 7,300 × in a Philips EM 300. The resulting 160 micrographs per series were contact-printed and the positive films projected on the screen of a table projector[6] at a final magnification of about 83,000 ×. The screen contained a square lattice test system, the distance between the points measuring 2 cm (equivalent to 0.241 μm on the section).

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[2] The authors wish to thank Professor E. R. WEIBEL for his interest in this work. We also thank Miss S. BLEIKER and Mr. K. BABL for their skillful technical assistance.
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Table I. Means and standard errors of V_{Vmi}*, V_{Vve}* and V_{Vli}* at 2 different magnifications (33'000×, 83'000×) of *T. brucei* STIB 33 BF

Series	1	2	3	4	5	6	7
f(SF)	0,13	0,24	0,25	0,48	0,89	0,98	1,0
V_{Vmi}*							
33'000×	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
83'000×	0,123 ± 0,009	0,143 ± 0,011	0,103 ± 0,006	0,078 ± 0,002	0,055 ± 0,002	0,051 ± 0,003	0,044 ± 0,003
V_{Vve}*							
33'000×	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
83'000×	0,076 ± 0,008	0,074 ± 0,005	0,082 ± 0,003	0,080 ± 0,004	0,089 ± 0,006	0,098 ± 0,003	0,064 ± 0,003
V_{Vli}*							
33'000×	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
83'000×	0,020 ± 0,002	0,021 ± 0,003	0,021 ± 0,0004	0,021 ± 0,002	0,025 ± 0,002	0,024 ± 0,003	0,015 ± 0,001

f(SF) = frequency of slender forms.

The volume densities were estimated with respect to the cytoplasm of the cell body (without flagellum = V_V*). The relative volumes of the mitochondrium (V_{Vmi}*), the vesicles (V_{Vve}*) and the lipid inclusions (V_{Vli}*) were obtained by point counting. 6 samples were evaluated for each series. Each sample represented an area of trypanosome-cytoplasm covered by 1,000 points, corresponding to an area of about 60 μm^2 per sample.

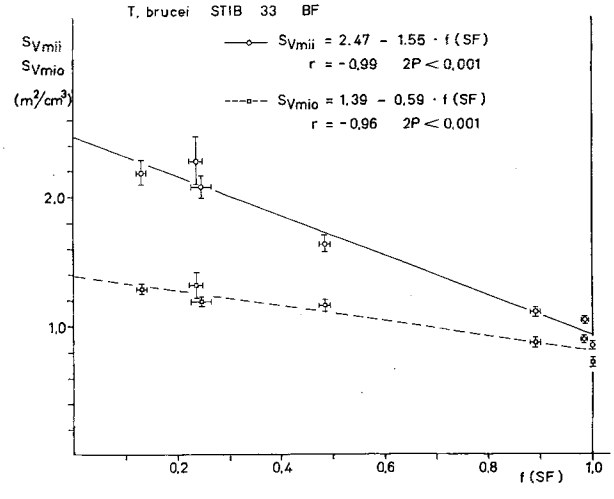
The surface densities of the inner (S_{Vmii}) and outer (S_{Vmio}) mitochondrial membranes were estimated by intersection counting between the membranes and the test lines. This was done in 2 directions perpendicular to each other. Surface densities (S_V) were calculated according to the basic formula: $S_V = 2 \cdot I_L$[6]. Furthermore the ratio of the inner to the outer mitochondrial membranes S_{Vmii}/S_{Vmio} and their surface to volume ratios (S/V) were calculated. Knowing the approximate absolute volume of the cytoplasmic cell body (without flagellum = Vcy*) for the slender and stumpy blood form[3], the absolute surface of the inner and outer mitochondrial membranes could be derived for these two types of *T. brucei*.

Table II. Absolute surface area of the inner and outer mitochondrial membranes in the slender and stumpy blood form of *T. brucei* STIB 33

	Slender form	Stumpy form
Inner membrane (S_{mii}) μm^2	19	136
Outer membrane (S_{mio}) μm^2	17	77
(Absolute volume of cytoplasmic cell body without flagellum μm^3)	(21)	(55)

Results. 1. *Volume densities* (Table I). Although the volume densities of the mitochondrium, the vesicles and the lipid inclusions have been determined in a previous paper[3], they were again evaluated in the present study at a higher magnification. This permitted the derivation of the surface to volume ratios for the mitochondrium. As a whole, the volume densities obtained at a magnification of 83,000× were systematically higher than those obtained at 33,000×.

2. *Surfaces of mitochondrial membranes.* The surface density of the inner mitochondrial membrane (S_{Vmii}) increases significantly from 0.93 m^2/cm^3 for slender forms to 2.47 for stumpy forms (Figure 1). The outer mitochondrial membrane surface density (S_{Vmio}) increases at a less steep rate from 0.81 to 1.39 m^2/cm^3 (Figure 1). The steeper increase in S_{Vmii} with decreasing f(SF) is also reflected by the ratio S_{Vmii}/S_{Vmio}, which is 1.18 for slender and 1.84 for stumpy forms (Figure 2). When absolute surfaces are calculated for these parameters (S_{mii} and S_{mio}), it appears that they do not differ markedly in the slender form (Table II). The values for the stumpy form, however,



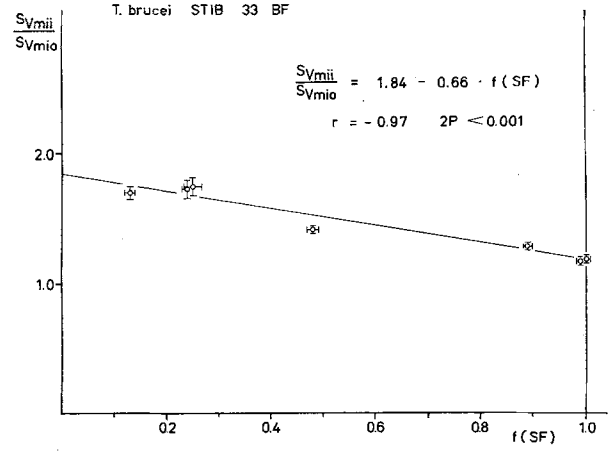


Table III. Means and standard errors of the surface to volume ratios for inner $(S/V)_{mii}$ and for the outer $(S/V)_{mio}$ mitochondrial membranes of *T. brucei* STIB 33 BF

Series	1	2	3	4	5	6	7
f(SF)	0,13	0,24	0,25	0,48	0,89	0,98	1,0
$(S/V)_{mii}$	18.0 ± 0.8	16.0 ± 0.8	20.5 ± 1.3	20.8 ± 0.6	20.1 ± 0.6	21.0 ± 0.9	19.7 ± 1.0
$(S/V)_{mio}$	10.7 ± 0.7	9.3 ± 0.4	11.8 ± 0.7	14.7 ± 0.4	15.7 ± 0.4	17.9 ± 0.7	16.7 ± 0.8

f(SF) = frequency of slender forms.

are higher and differ largely with 136 μm^2 for S_{mii} and 77 μm^2 for S_{mio}. This general increase and the difference between S_{mii} and S_{mio} are explained by the changes occuring in 3 parameters: V_{cy}*, S_{Vmii} and S_{Vmio}.

3. *Mitochondrial surface to volume ratios* (Table III). Surface to volume ratios can be calculated for the inner $(S/V)_{mii}$ and outer $(S/V)_{mio}$ mitochondrial membranes. Whereas $(S/V)_{mii}$ does not vary significantly for the 7 series, the $(S/V)_{mio}$ is decreased from 17.3 m^2/cm^3 for the slender to 9.3 for the stumpy form. These values cannot be read from the Table; they correspond to the extrapolated data obtained by the calculation of the regression line for $(S/V)_{mio}$, which is:

$$(S/V)_{mio} = 9.3 + 8.0 \times f(SF);$$

the correlation coefficient r is 0.94 and the attained significance level for 2 P is better than 0.005.

Discussion. In the present paper we found higher values for V_{Vmi}*, V_{Vve}*, and V_{Vli}* than those given in a previous publication[3]. These differences are due to the two magnifications used for the morphometric evaluation. The high magnification of 83,000 × allows a good recognition of even indistinct profiles of mitochondrium, vesicles and lipid inclusions. At the lower magnification one probably missed small sections of the above-mentioned organelles because of their low contrast against the cytoplasmic matrix. It is obvious that 'rare' organelles, such as vesicles and especially lipid inclusions, will be particularly stricken with this effect. On the other hand, one has to keep in mind that an improved detection of capsectioned organelles leads to an overestimation of their volume density. This factor alone, however, cannot account for the whole difference between the two estimations.

The fact that the use of a higher magnification may often yield higher volume densities on the same preparation seems to be a general phenomenon inherent to morphometric investigations and needs further clarification. This systematic aberration, however, is of little consequence for our investigations, since we are especially interested in the differences between the pleomorphic forms of *T. brucei*. These differences are not affected by the choice of the magnification.

The present results show that during the transformation of slender to stumpy forms, the volume density of the mitochondrium and the surface densities of mitochondrial membranes increase. This trend is even more marked for the absolute values of these parameters, since stumpy trypanosomes have a larger cytoplasmic body than the slender ones. The absolute outer mitochondrial membrane surface area is augmented by a factor of 4.5 during the transformation, the inner one by a factor of 7. This higher rate of increase in S_{mii} is also reflected by the rise in the quotient S_{Vmii}/S_{Vmio}.

Analysis of the absolute data of the mitochondrium (surface areas of membranes and volume) reveals that, during transformation of *T. brucei* from slender to stumpy form, the surface to volume ratio of the inner membrane $(S/V)_{mii}$ remains practically constant, whereas the outer membrane surface to volume ratio $(S/V)_{mio}$ shows a fall, which corresponds to a surface area increase at the 2/3 power of the volume increase. This indicates that the stumpy form mitochondrium is certainly larger but is not altered in its structural composition. This large mitochondrium of the stumpy form may be expected to be prepared to the changes known to occur in its respiratory activity[4,5,7].

Zusammenfassung. Morphometrisch wird gezeigt, dass die stumpfe Blutform («stumpy form») von *Trypanosoma brucei* ein grösseres Mitochondrium besitzt als die schlanke Form («slender form»). Die strukturelle Zusammensetzung des Mitochondriums bleibt annähernd gleich.

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Enzymes of Non-agglutinable Vibrios and Their Possible Role in the Development of Enterotoxic Factor

It is now believed that the pathophysiological changes that occur in cholera are due to an exotoxin elaborated by *V. cholerae* containing two factors, one enterotoxic factor (EF) responsible for the initiation of fluid accumulation in the gut of both human and experimental animals, and the other permeability factor (PF), capable of producing increased vascular permeability in the skin of experimental animals[1,2]. It was reported earlier that the enterotoxic factor could be developed in non-agglutinable vibrios (NAG) after animal passages[3]. It is also known