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

Enzyme activity[a] of the non-agglutinable vibrios before and after animal passage

Enzyme activity	Substrate	Strains				
		NAG 108	No. of passage (NAG 108) 2	3	4	In. Assam Kuki
Mucinase	Ovomucin	16	16	16	16	16
Protease	Casein	2.9	2.3	2.4	1.5	1.7
Lecithinase	Egg yolk	16	16	64	128	128

[a] Reciprocal of the highest dilution (detail in text).

that the virulence of a strain could be increased by animal passage[4]. However, the mechanism by which the organisms acquire factors regulating the increased virulence, are not understood. In view of this, studies were undertaken to compare the differences, if any, in the enzymatic activity between NAG before and after animal passage in comparison with agglutinable vibrio. The results are reported in this preliminary communication.

Material and method. The strain of non-agglutinable vibrio, NAG. CRC 108/68, was isolated from a patient. This strain was passaged in the ligated ileal loops of adult rabbits, the methods of which are detailed in previous communication[3]. *V. cholerae*, Inaba, Assam Kuki, the standard laboratory strain with known virulence, was also included in the experiment. Sonicated cell-free extracts of the test organisms from a 18 h surface culture were used for enzyme assay. Procedure for assay of mucinase was according to KUSAMA and CRAIG[5], and of protease and lecithinase according to LIU et al.[6].

Result. It is evident from the Table that there is no difference in mucinase and protease activity between the strains of non-agglutinable vibrio before and after animal passage and also between strains of non-agglutinable and agglutinable vibrios, However, there is significant difference in the lecithinase activity between the non-agglutinable strains before and after animal passage as well as between *V. cholerae* and pre-passaged NAG vibrios. It was observed that the lecithinase activity is increased 4-fold at the 3rd passage and reached 8-fold after the 4th passage, thereupon the lecithinase activity reached a level equilavent to lecithinase activity produced by the agglutinable vibrio, *V. cholerae*, Inaba.

Discussion. It has been observed previously that enterotoxic factor could be induced in non-agglutinable vibrios after serial animal passages[3] but the mechanism by which the enterotoxic factor increased still remains unknown. Present investigation indicates that lecithinase activity of non-agglutinable vibrios was increased in the passaged strains upto the level of a known virulent strain of *V. cholerae*.

There are a number of studies on the enzymes of *V. cholerae*, particularly on the enzymatic make-up, but the role of an enzyme in virulence or in relation to pathogenicity has not been worked out[7,8]. It was thought previously that mucinase is produced in low titre by non-agglutinable vibrios than *V. cholerae*[9] and it was further shown that virulence of *V. cholerae* is related to high titre of mucinase[11]. But it was also shown that mucinase does not bear any relationship to the virulence of the organism[10,11]. Present study indicates that the mucinase activity of non-agglutinable vibrios was the same as agglutinable strains and no difference could be observed on animal passage.

Lecithinase was thought to be characteristic for El Tor biotype of *V. cholerae*, as its possible role in haemolysis; however, their presence was also found in classical cholera vibrios[12]. Reports on the presence of lecithinase and its biological activity in NAG are lacking. Though the present study does not deal with the biological activity of the NAG vibrios, it may be presumed that increased lecithinase activity in the NAG strains after animal passage may play some role in the development of enterotoxic factor as observed earlier. Enzymatic role in the virulence, either alone or in combination with cell-free protein toxin in vibrios, remains to be evaluated.

Résumé. Sachant que le facteur entérotoxique peut être développé dans les vibrions non-agglutinables par transfer animal, nous avons déterminé les activités enzymatiques (mucinase, protéase, lécithinase) de ces vibrions. Après ce transfert l'activité lécithinasique a augmenté, et cette activité est semblable à celle d'un virus (*V. cholerae*). Nous supposons que l'augmentation du facteur entérotoxique est causée par celle de l'activité de la lécithinase.

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12 June 1972.

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[13] Thanks are due to Dr. A. MONDAL for help and Mr. MANZAR ALAM for his secretarial assistance.