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THE EFFECT OF SOME FACTORS ON THE CELL VOLUME OF RUMEN CILIATES

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The significance of the rumen ciliate fauna in the nitrogen conversion chain in the ruminant forestomachs is a question not conclusively solved. Efforts have been made to calculate the magnitude of the fauna, with the intention of estimating the amount of ciliate protein available daily to the host animal. Some of these calculations have been based on ciliate counting and geometrical computation of the ciliate cell volumes using the ellipsoidal form of the ciliates as basis for the computations (*Mowry & Becker* 1930, *Schumacher* 1962, *Harmeyer* 1963).

The sources of error inherent in the counting methods applied, have been thoroughly tested (*Boyne et al.* 1957, *Warner* 1962, *Harmeyer*), while factors affecting the cell volumes have received less attention.

Factors most likely to cause errors in the volume computations seem to be 1) osmotic effects on the cells, caused by dilution of the rumen sample with hypotonic liquids, 2) the possible shrinking effect of formol or other fixatives and 3) the practice of conceiving ophryoscolecid ciliates in general as rotation ellipsoid bodies rather than as bilaterally compressed organisms.

The intention with the present investigation was to test the significance of possible errors inherent in the above mentioned phenomena and practices.

MATERIAL AND METHODS

In testing the first 2 mentioned factors, rumen ciliates simultaneously obtained from the same host were subjected to 3 different treatments: 1) immediate volume determination, 2) determination after dilution of the rumen sample with water and 3) determination after formol fixation.

The rumen samples were obtained from 2 sheep in connection with rumen fistulation. Sheep no. 1 was starved overnight while sheep no. 2 was fed its usual morning ration, consisting of hay and grain, on the day of fistulation. From both sheep, 3 samples, consisting of about 200 ml well mixed whole rumen contents each, were taken. The first one was collected in a prewarmed (39°C) thermos-flask, the second one was diluted 1:1 with luke warm tap water and the third one was diluted 1:10 with 4 % formol.

The volume determinations were based on the ellipsoid formula, $V = \frac{L}{2} \times \frac{W}{2} \times \frac{T}{2} \times \frac{4}{3} \pi r^3$, where L = the mean cell length of the species concerned (excluding possible spination), W = the mean width = the dorso-ventral diameter measured at the widest part of the cell and T = the mean thickness = the transdiameter measured at the thickest part of the cell. In this part of the investigation however, no measurements of thickness were taken. The mean thicknesses were calculated from the width/thickness ratios of respective ciliate species given by *Kofoid & MacLennan* (1932).

The ciliate species studied were Diplodinium (Eudiplodinium) maggii Fior. and Diplodinium (Diplodinium) dentatum Schuberg from sheep nos. 1 and 2 and Epidinium ecaudatum (Fior.) f. ecaudatum Crawley from sheep no. 2 alone. From the differently treated samples, measurements of length and width on 40 specimens of each species were taken and the respective mean dimensions and the errors of the means were computed.

The measuring of cells from the undiluted (native) samples was performed immediately after sampling. The ciliates, though, were too motile to be measured, why the slide preparations had to be chilled for some minutes in a refrigerator before taking the measurements. The taking of measurements from the samples diluted with water was started 2 hrs. after sampling and the formol fixed cells were measured 15 days after sampling.

The possible error arising when bilaterally compressed ciliates

Table 1. Length and width (μm) of rumen ciliates in the native state, after water dilution of the samples and after formol fixation. The levels of significance of differences between the native and the treated states are shown.

Sheep no.	Ciliate		Native dimens.	2 hrs. H_2O	Level of significance P <	15 days formol	Level of significance P <
1	D. dentatum	L	75.5±1.17	82.0±1.44	0.001	77.4±1.41	no significance
		W	55.4±0.62	57.1±0.75	no significance	55.2±0.87	„
	E. maggii	L	146.1±1.85	153.7±2.42	0.01	147.2±2.47	„
		W	99.4±1.10	116.0±2.24	0.001	93.6±1.16	0.001
2	D. dentatum	L	88.9±1.39	97.7±1.64	0.001	81.3±1.20	0.001
		W	65.0±0.95	74.8±1.55	0.001	61.0±0.73	0.001
	E. maggii	L	146.9±1.96	167.3±2.53	0.001	147.4±1.68	no significance
		W	95.4±1.24	125.8±2.00	0.001	92.7±0.88	„
	E. ecaudatum	L	133.5±2.47	139.0±2.60	no significance	123.8±2.07	0.01
		W	64.4±1.18	86.5±1.66	0.001	62.8±1.48	no significance

are regarded as rotation ellipsoids was calculated on the basis of measurements taken on ciliates from reindeer. The species measured were Entodinium anteronucleatum Dogiel, Diplodinium (Diplodinium) dogieli Dogiel, Diplodinium (Eudiplodinium) spectabile Dogiel and Enoploplastron triloricatum (Dogiel). The number of measurements taken on each dimension from the respective species was not the same for all species concerned, but was larger than 100 for length and width and more than 50 for thickness. On the basis of mean dimensions derived, 2 different volumes for each species were calculated — 1 according to the rotation ellipsoid formula, where the transdiameter of the cell is conceived being the same as the dorso-ventral diameter, and 1 using the calculated mean transdiameter.

The ciliates were measured in unstained wet preparations. The image of each cell was projected through a camera lucida on a measuring table and measured with a scale copy of a stage micrometer. The magnification amounted to some 440 ×.

RESULTS

The effect on the cell dimensions obtained by dilution and formol fixation is shown in Table 1. The level of significance of the differences between the "native" state of the cells and the

Table 2. Calculated cell volumes ($\times 10^{-4}$ μm^3) of rumen ciliates in the native state, after water dilution and after formol fixation. The percentage differences between the native and the treated states are shown.

Sheep no.	Ciliate	Native	2 hrs. H_2O	% increase	15 days formol	% decrease
1	D. dentatum	9.7	11.2	15.4	9.7	0
	E. maggii	68.8	98.7	43.5	60.9	11.5
2	D. dentatum	15.6	22.6	44.8	12.5	19.8
	E. maggii	63.7	126.2	107.3	60.5	5.0
	E. ecaudatum	29.0	54.6	88.3	25.6	11.7
mean change in volume				+59.9		—9.6

states caused by dilution and fixation respectively is also shown. In Table 2, the calculated mean cell volumes of each species, obtained after the treatments mentioned, are shown, as well as the percentage difference between the "native" and treated volumes.

The volumes calculated according to the 2 different ellipsoid formulas, and the percentage differences between the results are shown in Table 3.

Table 3. Length, width and thickness (μm) of some rumen ciliates and the cell volumes calculated according to I) the ellipsoid formula and II) the rotation ellipsoid formula. The percentage difference between the results are shown.

Ciliate	Dimens.	Mean$\pm E_M$	Volume I: ellipsoid formula	Volume II: rotation ellipsoid formula	Difference in %
E. anteronucleatum	L	60.3±0.32	3.7×10^4 μm^3	5.7×10^4 μm^3	54
	W	42.5±0.17			
	T	27.6±0.29			
D. dogieli	L	103.9±0.38	17.8×10^4 μm^3	31.5×10^4 μm^3	77
	W	76.0±0.41			
	T	43.1±0.55			
D. spectabile	L	111.4±0.65	21.0×10^4 μm^3	35.0×10^4 μm^3	67
	W	77.5±0.52			
	T	46.5±0.83			
E. triloricatum	L	89.9±0.63	7.7×10^4 μm^3	10.4×10^4 μm^3	35
	W	47.1±0.29			
	T	34.6±0.45			

DISCUSSION

The practice of diluting the rumen samples with water 1:1 was used by *Mowry & Becker* (1930). Also *Ferber* (1928) determined the proportion of ciliates in the rumen contents in a way which involved a considerable dilution of the samples with water. The present investigation indicates that the ciliates absorb water from hypotonic solutions which causes them to swell. Consequently, determinations of volume or weight of the fauna which involve dilution of the rumen liquid with water, are apt to result in values considerably higher than the actual ones. The methodical error involved seems to be a significant one, and the fauna volumes presented by the above mentioned authors must be regarded too large.

The formol fixation seems to affect the cell dimensions less than the dilution with water. The mean effect on the calculated cell volumes was less than 10 %. It appears that the shrinkage as well as the above discussed swelling might to some extent depend on the feeding time of the host animal. Morphological details, such as the skeletal plates of the ciliates, might also influence the changes occurring in different dimensions of the cells. Because of the lack of uniformity in the changes observed, it seems unwise to draw conclusions about the magnitude of the errors caused by the treatments studied. Further investigations, including the testing of buffering methods, are required.

When the thickness of the cells was neglected, none of the computations performed revealed an error less than 35 % of the actual cell volume. The large discrepancy between the cell volumes obtained by the 2 different geometrical approaches, indicates that the calculations of cell volumes ought to be based on the actual thickness of the species studied, as well as on the length and width.

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SUMMARY

The effect of diluting rumen samples with water and that of formol fixation, on the volume of ophryoscolecid rumen ciliates, was studied. The dilution with water caused within 2 hrs. the ciliates to swell to an average of 60 % (15.4—107.3). During 15 days of formol fixation, the shrinkage was on an average 10 % (0.0—19.8) of the original volume.

When the thickness of the cells was conceived equal to the width, volumes 35—77 % too large were obtained.

The influence of these sources of error on calculations of rumen fauna volumes is discussed.

SAMMANFATTNING

Några faktorers effekt på våminfusoriers cellvolym.

I undersökningen studerades effekten av vattenspädning och formolfixering på volymen hos våmciliater av familjen Ophryoscolecidae. Spädning av våmproven med vatten fick cellerna att inom 2 timmar svälla med i medeltal 60 % (15,4—107,3). Formolfixering av proven i 15 dagar förorsakade en krympning på i medeltal 10 % (0,0—19,8) av den ursprungliga volymen.

När cellernas tjocklek sattes lika med bredden, erhölls 35—77 % för stora cellvolymer.

Dessa felkällors betydelse för beräkning av våmfaunans storlek diskuteras.

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