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THE CYTOPYGE OF CILIATA

II. MICROPHOTOGRAPHICAL DOCUMENTATION OF THE DEFECATION IN PRORODON TERES

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Abstract

The defecation in *Prorodon teres* was investigated in serial photographs. (1) The frequency of defecation and the number of faecal balls are in direct relation to food supply. As a rule only one faecal ball is discharged. (2) Defecation occurs almost regularly shortly before encystment, and immediately after excystment. (3) The faecal ball consists of numerous individual egestion vacuoles and it is as a rule approximately spherical. Exceptionally, a conic ball tapering anteriorly has been encountered. (4) The faecal ball covered by a distinct membrane usually splits several minutes after release as a finely granulated mass including various larger inclusions. (5) The excretion lasts 10 to 60 seconds on the average. (6) During defecation the pellicle closing the cytopyge opening (the cytopyge lock?) is resorbed and, at the same time, a new pellicle (new cytopyge lock?) is formed which then grows around the faecal ball from anterior to posterior. As the pellicle has completely enclosed the faecal ball, i.e., after the old pellicle had been resorbed, the faecal ball leaves the animal.

Introduction

Reviewing the cytopyge of *Ciliata*, the author has pointed to the difficulties concerning the investigation of this organelle [2]. The failures are due to the lack of photographical documentation concerning the defecation in *Ciliata*. Even the scarce, and partly contradictory, electron microscopic studies [1, 3, 8] have failed to clarify the mechanism of the cytopyge function. To my best knowledge, this is the first photographical documentation of the different phases of the defecation in a *Ciliata*.

Material and method

Prorodon teres (Fig. 1) is encountered in large number in the alga waddings of the border area of "Lange Lacke" in Burgenland. Several hours after catch the animals attach to the glass wall of the vessel and thus are easily available for investigation in large masses. It lasted several days until an active ex- and encystment ensued.

Depending on the quality of supply *Prorodon teres* takes up variable food: mostly bacteria, small algae and detritus. Sometimes oscillatoria were taken up the excretion of which met difficulties (FOISSNER, unpublished data). Accordingly, the composition and appearance of the faeces were also variable. A rapacious mode of life could not be observed.

Preparations on normal slides were examined by phase-contrast microscopy. The organisms were (1) free-moving (swimming coverslips) or (2) slightly trapped. In the latter case care was taken to exclude strong cohesion powers which might cause artificial changes (e.g., protracted defecation). The second method proved to be especially favourable for photographic documentation, for the animals were often intensively moving (rotatory movement) during examination. The results obtained by the two methods were consistent.

Results

Prorodon teres has proved to be a proper animal for such investigations, because (1) it forms well-recognizable faecal balls, (2) defecation regularly takes place after excystment and (3) in this animal the cytopyge is much more frequently working than in many other *Ciliata*, especially if food is available abundantly.

The cytopyge is located in *Prorodon teres*, like in almost all holotrichous *Ciliata*, posteriorly. It includes the whole posterior polar area approximately 45 μm in diameter. It is light-microscopically visible while being active. In fact it only reaches this size when a number of faecal balls are being pushed out one after another (Fig. 17).

The process in the course of which food vacuoles turn into egestion vacuoles, i.e., faecal vacuoles, has not been studied in detail in *Prorodon teres*. It is, however, well-known, due to light- and electron microscopic studies, in other *Ciliata* [e.g., 5, 8], and it must be essentially the same in this species. The faecal balls were strikingly large in size, many times as large as any of the food vacuoles in this organism.

The defecation itself, i.e., the release of the faecal ball, is subjected to a modification in *Prorodon*, mainly due to the quality and size of the faecal material to be discharged. The four examples documented in this paper are characteristic of the defecation in *Prorodon*.

Case A: Fig. 1 shows a free-swimming *Prorodon* containing a large faecal ball (arrow) immediately before being released. Besides, the posterior end of the animals is often tip-like prolonged especially if the animal is able to move freely. The spherical faecal ball is substantially larger than the largest food vacuole in the animal. It is limited by a well-defined membrane and possesses a very compact structure.

Case B: Figs 2 and 3 show two stages of the excretion of a faecal ball immediately after excystment. The conical, anteriorly tapering, form of the faecal ball is striking. During the process of discharge the cytoplasmic area over the ball showed a quick rotatory movement. Fig. 3 shows the faecal ball approximately half a second before the ball had been released. In contrast to the quick rotatory movement of the whole animal, the faecal ball has remained immovable; it is enclosed in a well-defined membrane. Ten minutes thereafter the ball split into a fine granular mass.

Case C: This series of figures (4—11) shows the process of excretion at approximately 3-second intervals from beginning to end. Thus, the whole process lasted about 25 sec. Since the animal was slightly trapped by the coverslip, it is possible that the duration of the defecation was somewhat prolonged. In a free-swimming organism the defecation as a rule reaches an end within 3 to 20 sec.

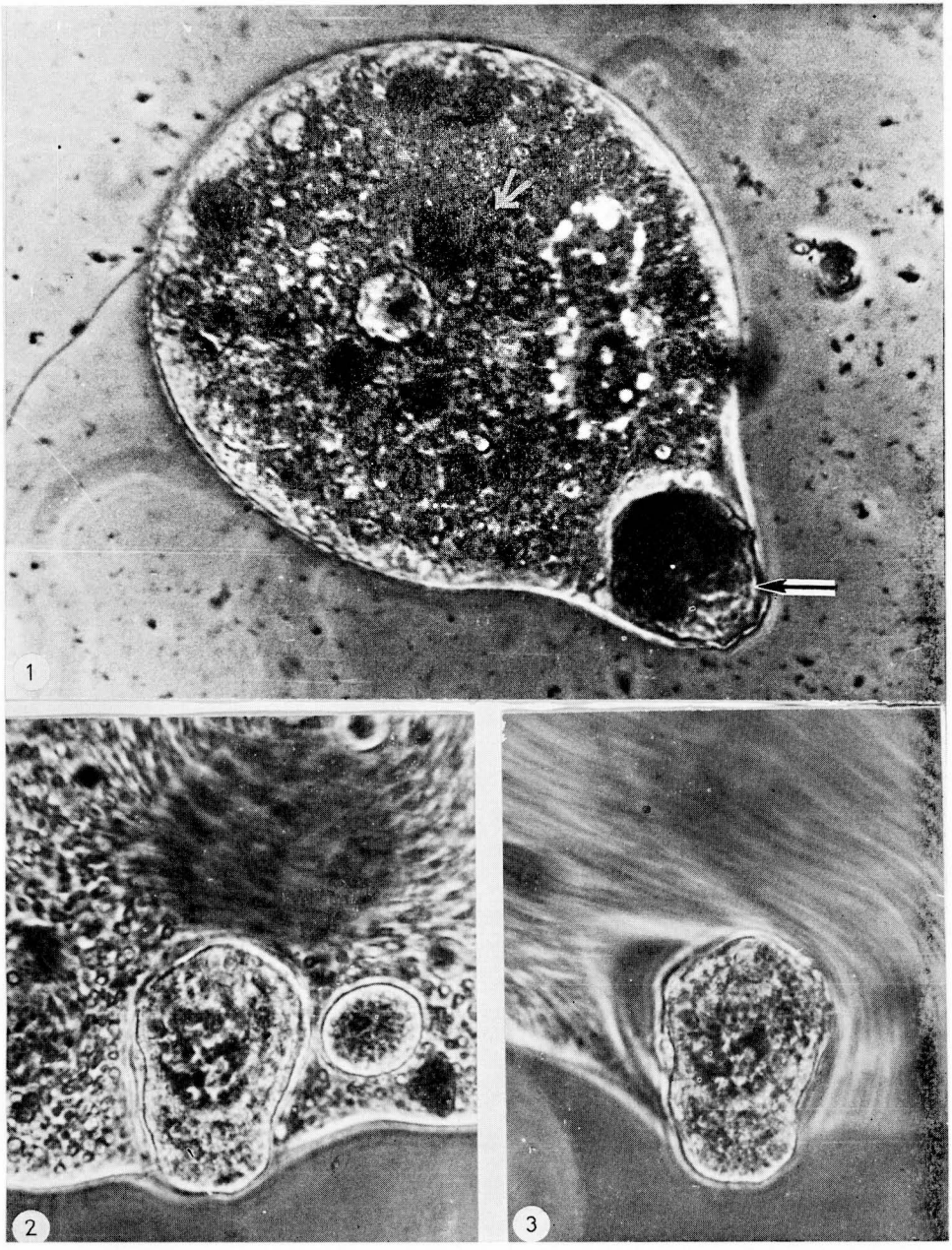
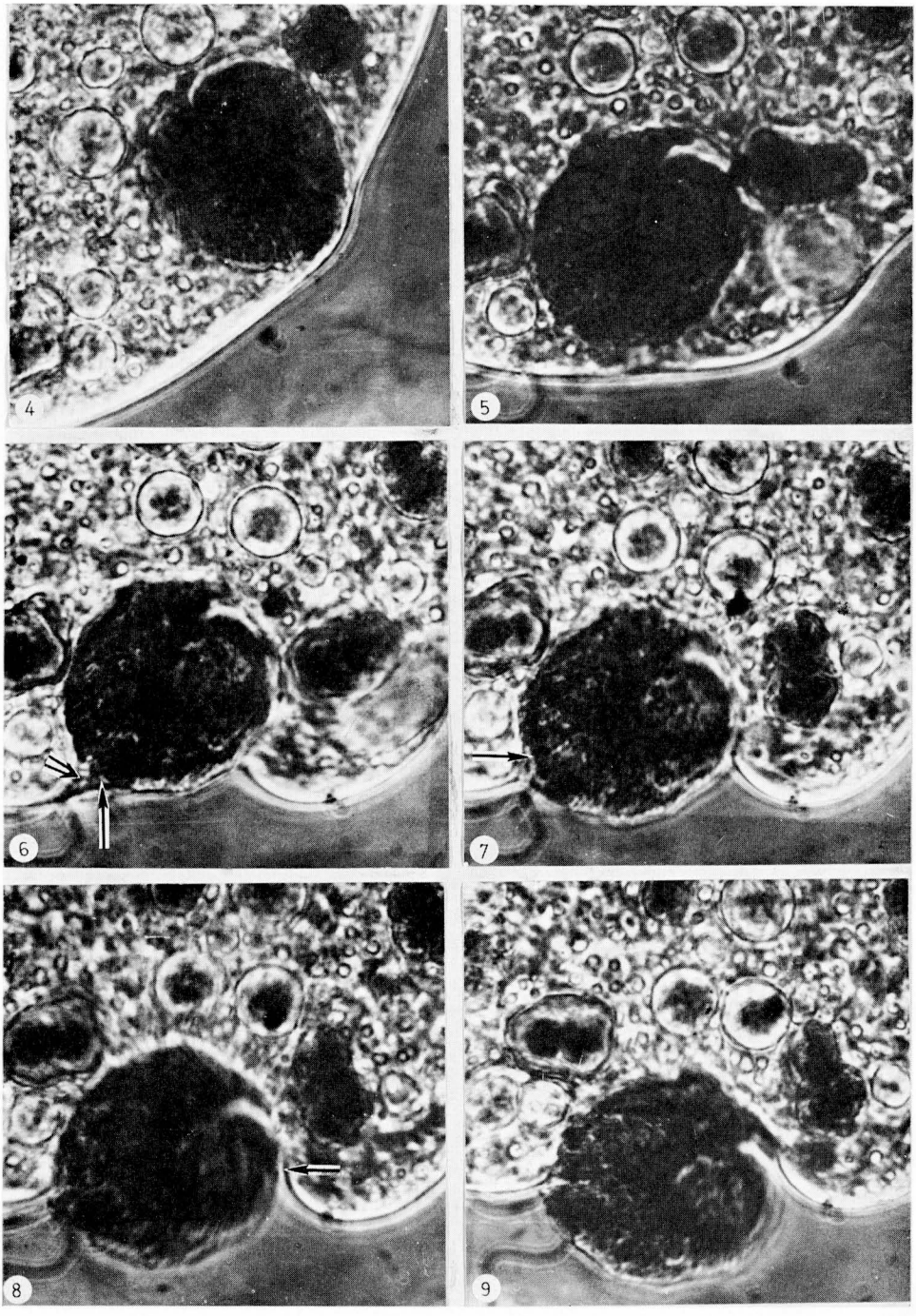
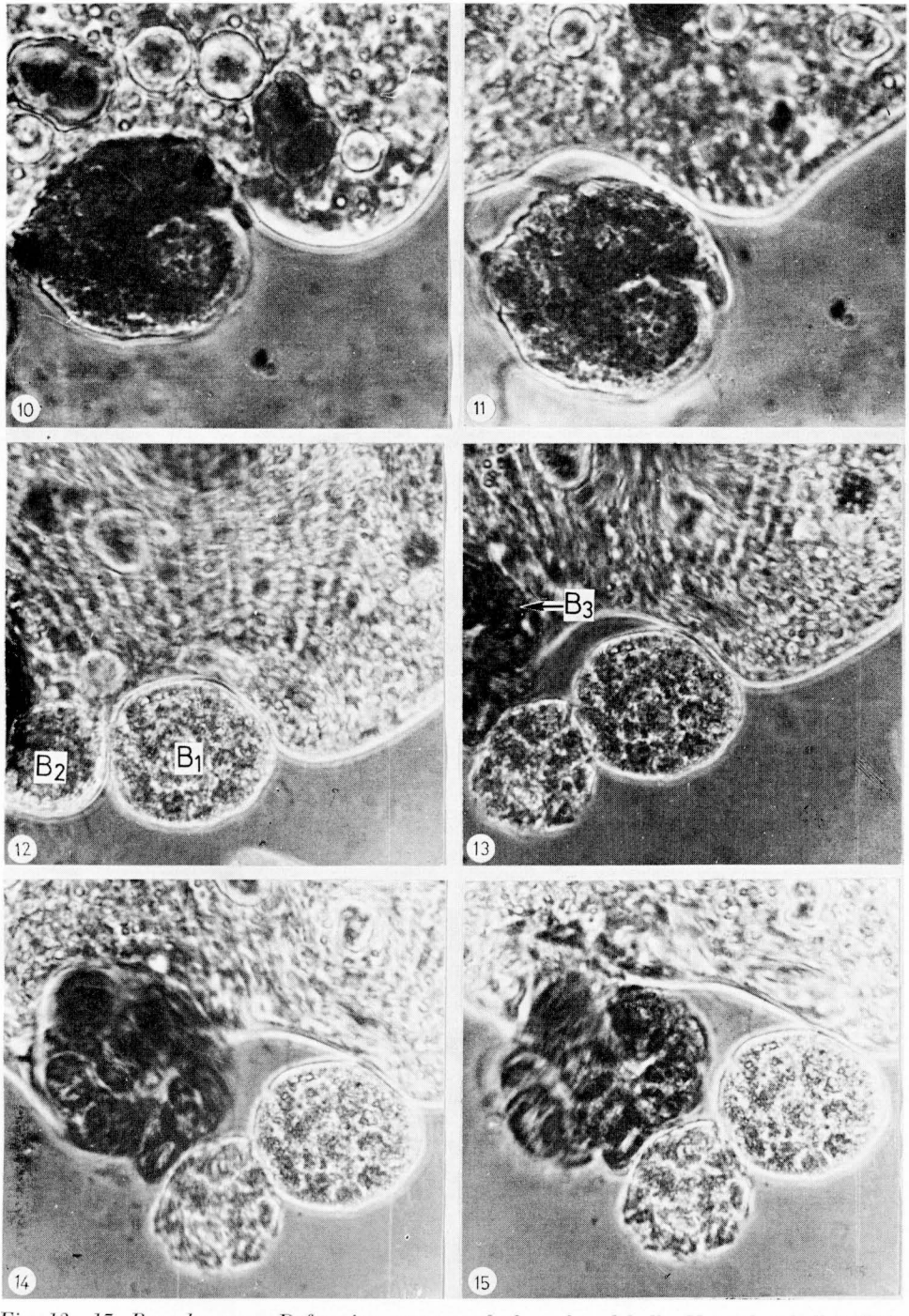


Fig. 1. Prorodon teres with a large faecal ball (arrow) and an early stage of defecation. Approx. ×500

Figs 2 and 3. Prorodon teres. Two consecutive stages of defecation of a faecal ball tapering conically anteriorly. For details, see the text. Approx. ×1 000

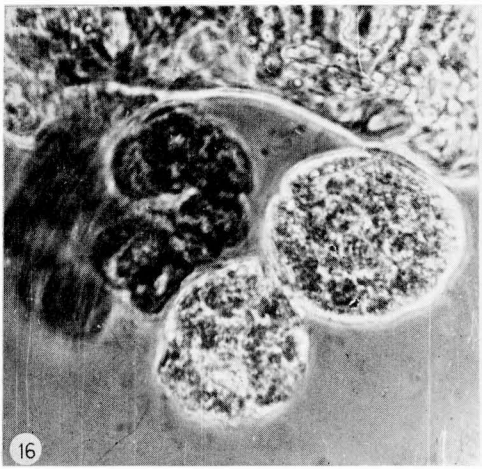


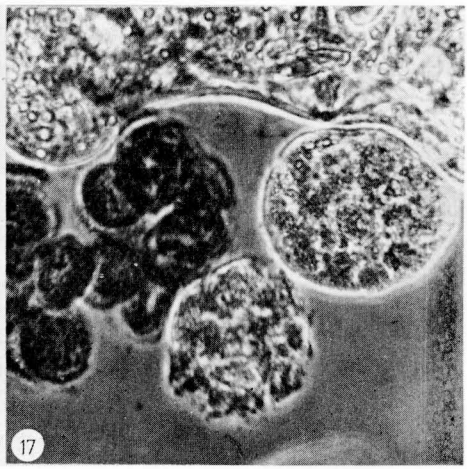
Figs 4—11. Prorodon teres. Defecation process of one faecal ball from the beginning to the end. For details, see the text. Approx. ×1 000



Figs 12—17. Prorodon teres. Defecation process of three faecal balls. Note that ball 3 (B3) consists of many small egestion vacuoles. For details, see the text. Approx. ×1 000

Stage 1 (Fig. 4). The internal wall of the pellicle is pushed by the faecal ball so that the former appears to be bulged outwards. *Stage 2* (Fig. 5). Due to the rotatory movement of the animal, the faecal ball has been shifted to the left. However, it has been pushed somewhat centrally, hence the pellicle is bulging inward. *Stage 3* (Fig. 6). For the present, the faecal ball does not move outwards; instead, the lower part of the animal begins to surround the ball so that a well-defined bulge appears on both sides. The behaviour of the part of the pellicle on the outer surface on which the faecal ball is situated is of special interest. On the left it is well seen that the pellicle is bulging from inside to





surround the faecal ball. The introduction of this process, i.e., the burst of the pellicle on the area where the ball leaves the animal, is well shown by the left side of the picture (arrow). The old pellicle with the membrane of the faecal ball still appears to be congruent. At the same time a new, very thin, pellicle (double arrow) appears which encloses the ball posteriorly.

This stage clearly shows that in *Prorodon teres* no real burst of the pellicle (the cytopyge lock?) occurs; instead, an active rearrangement of the pellicle ensues. During this process the pellicle closing the cytopyge is resorbed at every discharge and at the same time a new pellicle (new cytopyge lock?) is formed.

Stage 4 (Fig. 7). The bulge of the cytoplasm surrounding the faecal ball has grown larger and reached its highest size. On the left, the new pellicle is well recognizable (arrow) while only insignificant remnants of the old pellicle are present. The faecal ball is now partially out of the animal. *Stage 5* (Figs 8 and 9). The newly formed pellicle is already complete. It is clearly separated from the membrane of the faecal ball and surrounds the latter posteriorly (Fig. 8, arrow). The ball is gradually wandering out of the animal (Fig. 9). *Stage 6*

(Fig. 10). The greatest part of the faecal ball has left the animal; a rather compact membrane covering the ball is clearly recognizable. *Stage 7* (Fig. 11). The excretion has reached an end. The faecal ball, appearing as a spheric form of irregular outline, is already clearly separated from the animal. There are numerous larger well-defined inclusion bodies in the fine granular ground substance. At the site where the ball had left the body there is a well-recognizable pellicle light-microscopically undifferentiable from the normal pellicle. This is an unequivocal evidence of the formation of the new pellicle (new cytopyge lock?) while the faecal ball is leaving the animal.

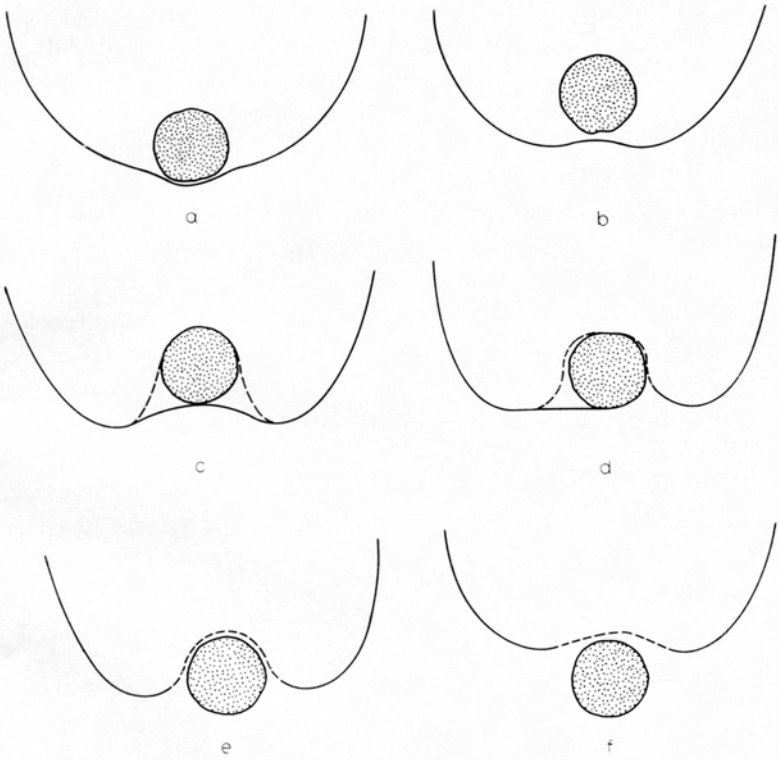


Fig. 18. Schematic representation of the defecation in *Prorodon teres*. Hatched areas indicate newly-formed areas of the pellicle. *a* = The faecal ball is pushed to the anterior side of the pellicle so that a bulge is formed. *b* = The faecal ball migrates inward in the animal and the pellicle begins to form a bulge inward. *c* = The bulge of the pellicle, starting posteriorly, is formed on both sides of the ball and grows around it. *d* = The old pellicle has almost completely been resorbed and the anterior half of the faecal ball is already out of the animal. *e* = The old membrane closing the cytopyge has been resorbed. The end of the faecal ball is already far from the animal. *f* = Defecation has been finished. The faecal ball, grown around by the new pellicle, has, so to say, fallen out of the animal. Immediately after the release of the ball, the newly formed area of the cytopyge membrane cannot be distinguished from the original pellicle: an evidence that the new cytopyge lock is formed during defecation.

Case D: In the cases described above only one faecal ball was discharged. If, however, feeding is abundant several faecal balls may follow one another. In the present case three balls were excreted within 60 seconds.

Ball 1 (Fig. 12, B1) had been discharged at the time of taking the first photograph. Since, however, the animal's moving was inhibited by a large detritus, it did not change its place and the ball is still tightly lying on the new pellicle. Left to ball 1, ball 2 is seen (Fig. 12, B2) immediately before being discharged; it is substantially smaller than ball 1. By the following stage (Fig. 13), the animal had moved sideways making ball 1 free. Ball 2 has just been pushed off. The time interval between the two photos was approximately 5 seconds. Ball 3 (Fig. 13, B3), following ball 2, just begins to be pushed out of the organism. Fig. 14 shows the completely discharged balls 1 and 2 and ball 3 being partially discharged. In Fig. 17 all the three balls have already left the organism. The new pellicle at the site of discharge is well visible.

Besides the difference in size, the different consistency and the different appearance of the faecal balls are striking. Ball 1 which was the first to be discharged was still unaltered when balls 2 and 3 have already begun to dissolve. Ball 3 was split into small spherical subunits immediately after having been discharged. The splitting of faecal balls is of interest because it allows us to draw some conclusions concerning the formation of the faecal ball. As it has already been mentioned the faecal balls often exceed the food vacuoles in size. It may therefore be concluded that the food vacuoles are not defecated one by one, but they coalesce to form a large egestion vacuole and are thus discharged. In faecal balls 1 and 2 the egestion vacuoles are still well recognizable: darker centres are surrounded by lighter areas. These correspond to food vacuoles.

The fact that in ball 3 the individual egestion vacuoles were not yet united into a single compact faecal ball might be attributed to the abundant food supply and a consequent poor digestion and compression of food remnants.

Discussion

The cytopyge mechanism of Prorodon teres

The above-described defecation processes in *Prorodon teres* leave no doubt that at the site of discharge of the faecal ball the pellicle (i.e., the membrane closing the cytopyge) is resorbed and, at the same time, a new pellicle, growing posteriorly around the faecal ball, is formed. The excrements consequently fall out the animal. The whole process resembles an exocytosis. The defecation is schematically illustrated in Fig. 18.

It is not supported by any evidence that the faecal material is pushed out of the animal by mechanical power. That the discharge is sometimes

eruption-like, might be attributed to an increased difference in the hydrostatic pressure between the body of the animal and the surrounding medium.

It is not clear why there is a close contact between the faecal ball and the pellicle during the earliest stage of excretion (see p. 358 Fig. 4), and why the ball is pulled back soon thereafter (Fig. 5). Hypothetically, an impulse by the faecal ball on the membrane closing the cytopyge might initiate the excretion. The fixed localization of the cytopyge provides another problem: there is no light-microscopical structure which might be responsible for that. Recent electron microscopic studies on *Paramecium* [1] have revealed fibrils running to the cytopyge conically, interpreted as receptor organelles for faecal balls. All these suggest that the defecation mechanism of *Prorodon teres* cannot be explained by a single restructuring of the pellicle: other structures and/or mechanisms must contribute to the process.

Coalescence of numerous egestion vacuoles to form a faecal ball

KAHL [4] was the first to report on numerous egestion vacuoles coalescing and subsequently being discharged together. Electron microscopic investigations on *Colpoda maupasi* [7] and *Tokophrya infusionum* [6] have likewise shown coalescence of food vacuoles in various stages of digestion. The present observations have led to the same conclusion. Neither the enormous size of the faecal ball nor the possibility of a splitting of the ball into sharply limited small subunits allows us to arrive at another conclusion. Nevertheless, there are other *Ciliata* in which each food vacuole is excreted separately (FOISSNER, unpublished data).

The significance of the large size of the faecal ball in the case of *Prorodon teres* is well understandable if it is taken into consideration that in a medium abundant in food 1 or 2 food vacuoles are formed per minute. Separate excretion of each vacuole would maintain a continuous flow of faecal material, and such a complicated process would mean a considerable charge for the animal.

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