Allometric growth of the proglottids and strobila of the tapeworm, *Hymenolepis diminuta*

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Abstract

Hymenolepis diminuta adults were killed and fixed in fully extended positions, and the total lengths of the strobilae, numbers of proglottids, and distances of proglottids along the strobilae were determined. The relationship of proglottid number to distance along the strobila was exponential. Beginning at proglottid 100 (P100), the lengths and widths of proglottids at 100 proglottid intervals were determined, and the surfaces areas were calculated. The relationships of proglottid length and width to proglottid number were linear, but the relationship of proglottid number to surface area (SA) was exponential. The volumes of proglottids were calculated, and the relationship of volume (V) to proglottid number was exponential. The relationship of surface area to volume ratio (SAVR) to proglottid number was exponential; at the anterior end of the worm (P100), the SAVR was 14.6, while at the posterior end of the worm (P1300) the ratio was 4.2. A single exponential equation describing the relationships among proglottid number, SA, and V was derived.

Introduction

In the definitive rodent (rat) host, the tapeworm, *Hymenolepis diminuta*, grows in a highly predictable fashion. Once a metacestode (cysticercoid) is ingested by the rodent host, growth of the tapeworm, as measured by increases in total weight, total length, or total number of proglottids, is exponential for approximately 7 days. Following exponential growth, the growth rate decreases until, at approximately 17–18 days post-infection (p.i.), gravid proglottids are shed at the same rate as new proglottids are formed, and the tapeworm no longer increases in length (reviewed by Roberts, 1980). The adult tapeworm, therefore, consists of immature proglottids at the anterior end that are very small and growing exponentially, and gravid proglottids at the posterior end that are much wider and longer. Between the immature and gravid proglottids is a continuous gradient of proglottids of increasing lengths and widths, so the tapeworm's strobila represents a gradient of proglottids with increasing surface areas and volumes.

Adult tapeworms lack a digestive tract. The tegument is the only interface between the parasite and its environment (the host's digestive tract), and all solutes entering and exiting the tapeworm must cross the tegument. Moreover, since adult tapeworms have no organs or organ system(s) that could be construed as being circulatory in nature, movement of solutes through the tapeworm's extracellular fluids probably occurs by diffusion only. Thus, the rate at which a tapeworm absorbs solutes from the external medium, and the distribution of these solutes along and within the tapeworm's body (strobila), must be determined in part by the ratio of the surface area (of the tegument) to the volume (of the proglottid). As noted above, the surface areas and volumes of proglottids increase as proglottids age, but how these parameters change and the relationship between them are unknown. Considering the potential importance of these parameters to our understanding of many different aspects of the biology of cestodes, ranging from growth and development to membrane biogenesis, the current study was undertaken to describe the relationship between

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surface area and volume along the strobila of *H. diminuta* and to test the following hypotheses: (i) the shape ('flatness') of the tapeworm proglottid results in a favourable (high) surface area to volume ratio (SAVR); (ii) the SAVR changes in a predictable manner as the proglottids grow.

Materials and methods

The OSU 'strain' (Pappas & Leiby, 1986) of *H. diminuta* was reared in grain beetles (*Tenebrio molitor*) and male Sprague-Dawley rats. Male rats (80–100 g) were infected with 30 metacestodes (cysticercoids), and the tapeworms were recovered 20–25 days p.i. Tapeworms recovered from four infected rats were used in this study. The rats were infected on different days and using different batches of cysticercoids, and the mean number of tapeworms recovered was 26. Tapeworms that were knotted or broken were discarded, and the remainder were killed, fixed, and mounted on slides (see below). For each set of measurements, at least two tapeworms from each rat were examined, and additional tapeworms for study were selected randomly from the remaining slides. The actual number of tapeworms used for each set of measurements is indicated in the results section.

After removal from the host's small intestine, the tapeworms were rinsed in several changes of buffered saline (KRT of Read, Rothman & Simmons, 1963), killed in an extended position by 'swirling' them in hot (55°C) water, and cut into pieces approximately 4 cm in length. The pieces were straightened and fixed for 12 h in buffered (100 mM phosphate, pH 7) 10% formalin. The pieces of strobila were rinsed in distilled water, stained overnight in diluted Gill's haematoxylin (Fisher Scientific, 1:4 haematoxylin:water), destained in acid alcohol, dehydrated, cleared in terpineol:toluene (1:1) followed by toluene, and mounted in Permount (Fisher Scientific) on glass slides. Various measurements of the tapeworms' strobilae were made. The total length of the tapeworm and the total number of proglottids were determined, and the lengths and widths of specific proglottids were measured; the latter measurements were used to estimate the surface areas of proglottids. The anterior or youngest proglottids tended to be rectangular, while the mature and gravid (older) proglottids were trapezoidal. The total surface areas of rectangular proglottids were estimated using the formula: A = (2)*(L*W), and the total surface areas for trapezoidal proglottids were determined as indicated in fig. 1. Measurements were made directly from stained, mounted tapeworms using an ocular micrometer, or by measuring digitized images of the tapeworm.

To estimate the volume of proglottids, it was necessary to measure their cross-sectional areas. For these measurements, slides containing pieces of stained tapeworm (i.e. whole-mounts) were soaked overnight in toluene to remove the cover slips. The stained pieces of tapeworm were rinsed in an additional change of toluene and embedded in Paraplast (Fisher Scientific). By embedding in paraffin relatively long pieces of stained strobila, it was possible to trim and mount the paraffin blocks so that the pieces of strobila were perpendicular to the microtome knife and, therefore, that cross-sections of the strobila were obtained. The paraffin blocks were sectioned at 15 μm, and the sections were affixed to subbed (gelatin-coated) slides (Pappas, 1971). The slides were rinsed in toluene to remove the paraffin, and cover slips were applied with Permount. The widths and depths (thicknesses) of numerous cross-sections were measured, and the cross-sectional area of each section was estimated as indicated in fig. 1.

For each relationship examined, the data were plotted separately using linear and exponential plots, and the regression equations and correlation coefficients were determined. In some instances the relationships between parameters were nearly linear and explained equally well using either linear or exponential equation (i.e. the r^2 values were nearly identical and very close to 1). In the results section, linear plots for these relationships are shown. Other relationships were, however, clearly exponential, and exponential plots of these relationships are shown in the results section.

Results

It was difficult to determine exactly where in a tapeworm's strobila the first proglottid was located. In the anterior section of a stained tapeworm, the first proglottids appeared as dark 'bands' in the strobila, but external demarcations of these youngest proglottids were not apparent. Moreover, these first few 'bands' were difficult to resolve from each other, and the ability to differentiate the 'bands' varied with staining intensity.

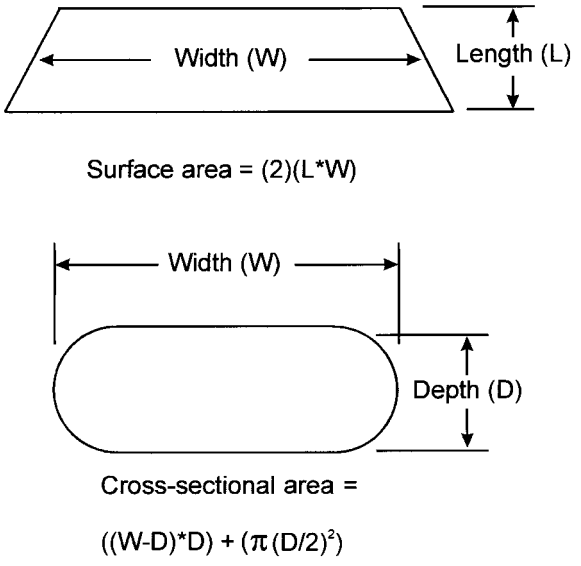

Fig. 1. Measurements taken and calculations of areas for the proglottids of *Hymenolepis diminuta*. For a rectangular proglottid (not shown), the surface area was calculated using the formula: SA = (2)*(W*L). For a trapezoidal proglottid (top), the surface area was calculated using the same formula, except that the width at the midpoint along the proglottid's length was used. Note that these formulae include both sides of the proglottid but assumed that the proglottid was flat. The bottom drawing shows the measurements taken and formula used for calculating the cross-sectional areas of proglottids.

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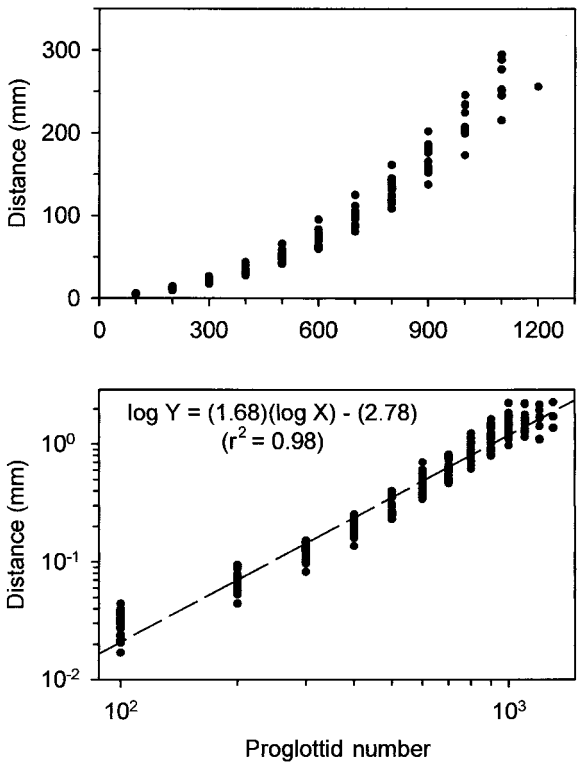

Fig. 2. The relationship between proglottid number and distance along the strobila of *Hymenolepis diminuta*. Adult tapeworms (n = 10) were killed in an extended position, and the distance of proglottids along the strobilae from the anterior end (scolex or holdfast) were measured. The individual points for each proglottid number represent the measurements for the individual tapeworms. Since some tapeworms had fewer proglottids than others, there are fewer data points for the higher proglottid numbers. In the top graph, the data are plotted on linear axes. In the bottom graph, the data are plotted on log axes, and the regression equation for the exponential relationship is included.

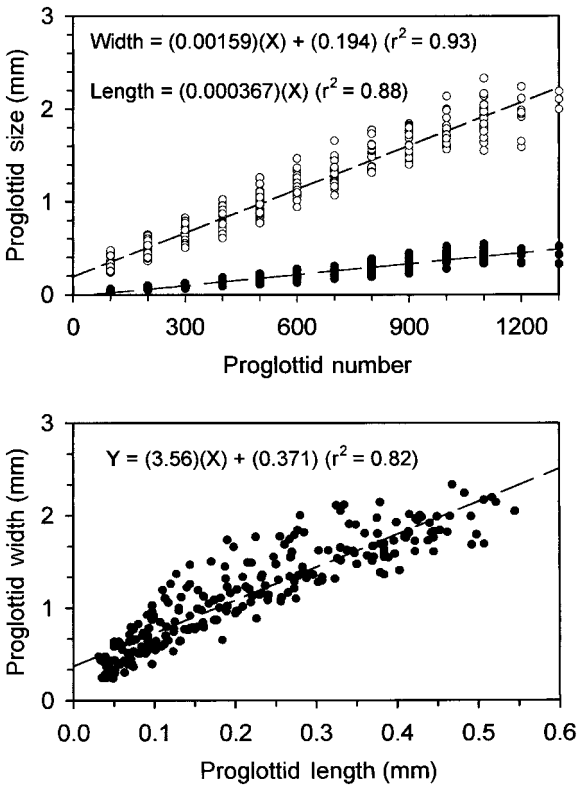

Fig. 3. The relationship between proglottid size and proglottid number along the strobila of *Hymenolepis diminuta*. The top graph shows the sizes of proglottids (widths ○; lengths, ●) versus proglottid number plotted on a linear scale. The proglottid widths represent the width at the midpoint along the proglottid's length. The data summarize the measurements taken from 21 tapeworms, 237 total proglottids. Since some tapeworms had fewer proglottids than others, there are fewer data points for the higher proglottid numbers (e.g. only three measurements at 1300). The regression equations are indicated in the graph. For the regression equation for proglottid length (●), the regression line was 'forced' through zero (as explained in the Results). The bottom graph summarizes the relationship between the lengths and widths of individual proglottids (237 proglottids from 21 tapeworms). The regression equation for the line is indicated in the graph.

Thus, in each tapeworm the first visible 'band' was counted as proglottid number 1 (P1). The proglottids immediately posterior to P1 were counted, but not measured, since errors in identifying P1 would have had a significant impact on the numbers assigned to these proglottids. For example, if P1 was incorrectly identified with an error of even five proglottids, there would be a 10% error in the determination of P50. The first proglottid measured was P100, An error of five proglottids in determining P1 would have resulted in only a 5% error in determining P100, and, in the more posterior parts of the tapeworm's strobila, the impact of this possible error would diminish (e.g. 1% at P500 and 0.5% at P1000).

The growth of *H. diminuta* is determinate. When the tapeworms become gravid (at approximately 18 days p.i.), they produce new proglottids and shed gravid proglottids at the same rate, and they do not increase in length. Thus, the sizes of individual tapeworms and the numbers of proglottids in individual tapeworms of comparable age were similar. A sampling of the heat-killed (fully extended) tapeworms used in this study yielded a length of 265 ± 22 mm and 1159 ± 118 proglottids (mean ± S.D., n = 20). When the distances of proglottids along the tapeworms' strobilae were compared, the relationship between proglottid number and distance was clearly exponential (fig. 2). The combination of data presented in these two figures indicated that there was little variability among the tapeworms recovered from different hosts.

The relationships between the widths and lengths of the proglottids versus the proglottid number were linear (fig. 3). Since the formation of proglottids begins some distance (≈ 1.5 mm) posterior to the tapeworm's holdfast, the positive Y-intercept for the equation describing the relationship between proglottid width and proglottid number was not unexpected. The equation for the

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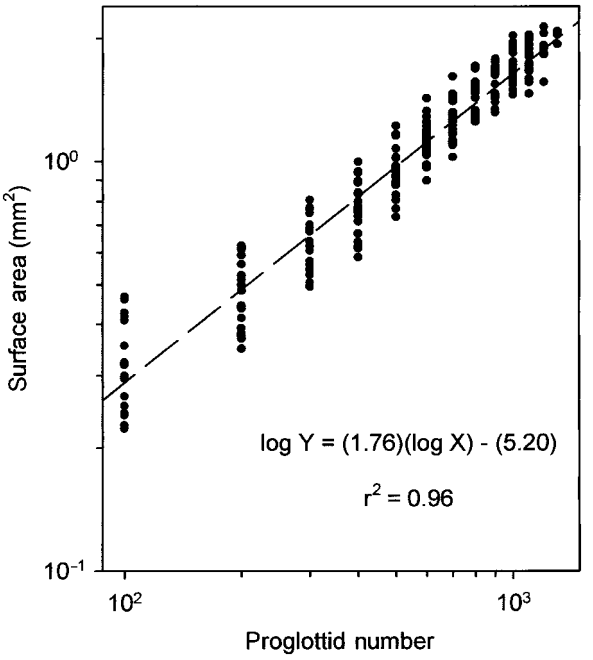

Fig. 4. The relationship between the surface area and proglottid number along the strobila of *Hymenolepis diminuta*. The surface areas (see fig. 1) of 237 proglottids from 21 tapeworms were determined, and each point represents a single proglottid. The regression equation is indicated in the graph.

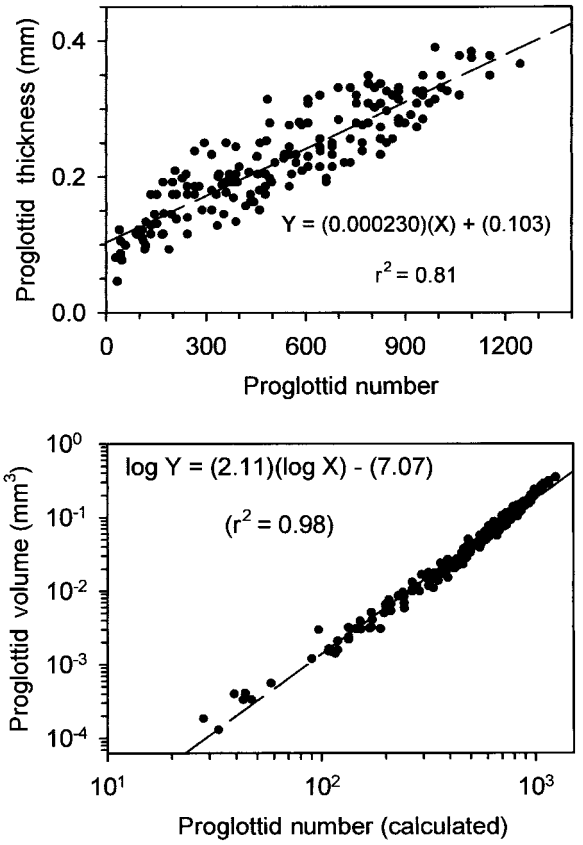

Fig. 5. The top graph shows the relationship between proglottid thickness and proglottid number along the strobila of *Hymenolepis diminuta*. Cross-sections of 176 proglottids were obtained from randomly selected sections of strobilae from 12 tapeworms. The thickness of each proglottid was measured, and the regression equation in fig. 3 was used to calculate the thickness of each proglottid. The regression equation for the relationship of proglottid thickness to proglottid number is indicated in the graph. The bottom graph shows the relationship between proglottid volume and proglottid number. Using the calculated proglottid number from the top graph and the regression equation in fig. 3, the length of each proglottid was calculated. The volume of each proglottid was then calculated as the product of the measured cross-sectional area and the calculated length. The regression for the line is indicated in the graph.

relationship between proglottid length (Y) and proglottid number (X) was $Y = 0.000389(X) - 0.0174$. Clearly, this equation did not describe accurately this relationship, since the negative Y-intercept would require that some proglottids be less than 0 mm in length. Thus, this line was 'forced' through zero, resulting in an equation of $Y = 0.000367(X)$ (a difference in slope of only 5%). A comparison of the slopes for the lines describing the widths and lengths of proglottids as functions of proglottid number indicated that the proglottids increased in width approximately four times faster than they increased in length. This observation was verified when the widths and lengths of individual proglottids were correlated. That is, the relationship was nearly linear, and the slope of the line (width/length) was 3.56 (fig. 3). Even though the relationships between widths and lengths of proglottids and proglottid number were linear, the widths and lengths increased at different rates. Thus, the relationship between proglottid number and surface area was exponential (allometric) (fig. 4).

To determine the volumes of proglottids, it was necessary to determine first the relationships among proglottid width, thickness, and cross-sectional area. Using cross-sections of proglottids, the widths and thicknesses of proglottids were measured (fig. 1). Using the regression equation describing the relationship between proglottid width and proglottid number (see Fig. 3), the proglottid number of each of the cross-sections that was measured was calculated. Readers should note that there were obvious, inherent errors in these calculations, especially in larger (more posterior) proglottids, because the larger proglottids were wider at the posterior end. Thus, if a particular cross-section was through the posterior end of a proglottid, the calculated proglottid number would overestimate the actual number. Similarly, if the cross-section was through the anterior end of a proglottid, the calculated proglottid number would underestimate the actual number. This may explain why these data (fig. 5) were more variable than those data for proglottid widths and lengths. Nevertheless, analysis of the data demonstrated a high linear correlation between the proglottid thickness and proglottid number (fig. 5).

Using this calculated proglottid number and the expression describing the relationship between proglottid

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length and proglottid number (see fig. 3), the length of the proglottid from which each cross-section was taken was calculated. The total volume of each proglottid was then calculated by multiplying the cross-sectional area by the calculated length of the proglottid. The relationship between proglottid number and volume was exponential (allometric) (fig. 5).

Using the same pieces of strobila as above (i.e. the cross-sections), the calculated proglottid number and the expression describing the relationship between proglottid number and surface area (fig. 3) were used to calculate the surface areas of the proglottids. The surface area to volume ratios (SAVR) were calculated and plotted against the proglottid number. There was an exponential (semi-log) gradient in the SAVR along the strobila, ranging from approximately 14.6 at P100 to 4.2 at P1300 (fig. 6).

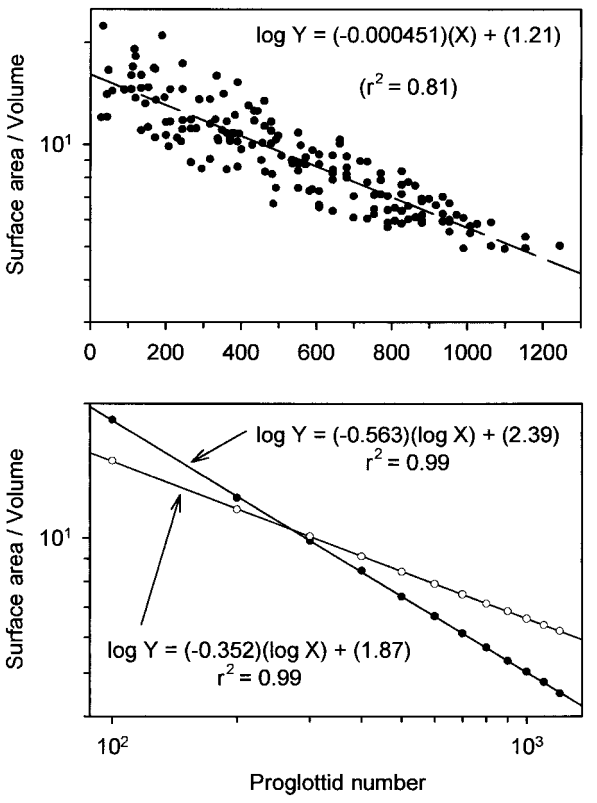

Fig. 6. The top graph shows the relationship between surface area to volume ratio and proglottid number along the strobila of *Hymenolepis diminuta*. Each point (n = 176) represents the ratio obtained by measuring the cross-section of a proglottid and then calculating its surface area and volume. The regression equation for the relationship is indicated in the graph. The bottom graph is a comparison of the surface to volume ratios of proglottids along the strobila of *H. diminuta* assuming the proglottids are rectangular (○) or circular (●) in shape and have equal volumes and lengths. The regression equations in fig. 3 were used to calculate the widths and lengths of proglottids at 100 proglottid intervals and the surface areas were calculated from these data. For each rectangular proglottid, the radius of a cylinder having the same volume and length was calculated. This radius was used to calculate the circumference of the cylinder, and the surface area was calculated as the product of the circumference and the length. The regression equations for the lines are indicated in the graph.

The relationships among proglottid number (PN), surface area (SA), and volume (V) were allometric. The regression equation ($r^2 > 0.99$) describing the relationship among these parameters was:

$$PN = (2.95) - (0.0016)*(\log V) + (0.57)*(\log SA) + (0.00061)*((\log V)*(\log SA))$$

Tapeworm proglottids are 'flat', and this may be an adaptation for maintaining a high surface area to volume ratio. To determine what effect 'round' (cylindrical) proglottids would have on this ratio, the surface areas of cylindrical proglottids having the same lengths and volumes as regular (flattened, rectangular) proglottids were calculated. That is, the appropriate regression equations were used to calculate the lengths (L), volumes (V), and surface areas (SA) of P100 through P1200 at 100 proglottid intervals, and the surface areas of cylinders having the same lengths and volumes as these proglottids were calculated. For P500, for example, L = 0.184 mm, SA = 0.356 mm^2, and V = 0.0423 mm^3. For P500 to be cylindrical and to have the same L and V, it would have to have a diameter of 0.541 mm and, therefore, a surface area of 0.312 mm^2. The surface area to volume ratios, as a function of proglottid number, for 'flat' and 'round' (cylindrical) proglottids were exponential, but the slopes of these relationships were different (fig. 6). Thus, at the anterior end of the tapeworm a 'cylindrical' proglottid would have a higher SAVR than a rectangular proglottid with the same length and volume, while just the opposite was true for proglottids at the posterior end of the tapeworm.

Discussion

The data of this study demonstrate clearly that growth of *H. diminuta* is allometric. The relationships of proglottid number (PN) versus distance along the strobila, surface area (SA), and volume (V) are exponential, and a single exponential equation describes the relationships among PN, SA, and V. Nevertheless, these data and equations must be interpreted with some caution for several reasons. First, the tapeworms used in this study were killed in an extended position by 'swirling' them in hot water, so the data reported herein probably represent the maximum surface areas and volumes of proglottids. Living tapeworms are considerably smaller than 'heat-killed' tapeworms (75–100 mm versus >250 mm), so these data may not be applicable to living tapeworms. Nevertheless, the data do provide good estimates of how the strobila and proglottids grow, and how the surface areas and volumes of proglottids change.

A second reason for caution is that the surface areas of proglottids were calculated assuming that the proglottids are flat. Thus, the contribution of the edge of a proglottid to its surface area is not included in the data, and ignoring this contribution results in a surface area that underestimates the actual value. Moreover, the magnitude of this potential error may vary along the tapeworm's strobila, since the anterior-most proglottids tend to have

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distinct 'edges', while the edges of the more posterior proglottids tend to be less distinct or rounded.

A third reason for caution is that the tapeworms used in this study came from rats infected with 30 cysticercoids. Because of the 'crowding effect', tapeworms in rats infected with fewer tapeworms will: (i) grow faster; (ii) reach a larger size; and (iii) contain more immature proglottids than tapeworms in rats infected with more tapeworms (Roberts, 1980). The lengths, widths, surface areas and volumes of proglottids of tapeworms from different infection intensities have not been measured, so it is not known if the mathematical relationships for tapeworms from 30-worm-infections apply to tapeworms from other infection intensities. However, it is reasonable to assume that, regardless of the infection intensity, the same paradigm applies to the growth of *H. diminuta*, i.e. growth is allometric.

The surfaces all of cestodes, including *H. diminuta*, are covered with microtriches. These bear a striking morphological resemblance to microvilli, and it is assumed that microtriches provide an increased surface area through which tapeworms can absorb materials from the surrounding medium. Thus, the sizes (lengths and diameters) and distributions (densities) of microtriches along a tapeworm's strobila are probably important in determining the 'effective' absorptive area. Threadgold & Robinson (1984) calculated the densities of microtriches (number μm^{-2}) and the 'functional amplification factor (FAF)' (i.e. the increase in functional membrane surface area) in the 'neck', 'mature' and 'gravid' regions of the strobila of *H. diminuta*. These authors were unable to assign specific numbers to the proglottids that were examined, and the values for these parameters were quite variable and failed to demonstrate a clear trend in the sizes and distributions of microtriches along the tapeworm's strobila. If the sizes and distributions of microtriches along the tapeworm's strobila do not change, as suggested above, then the FAF would not change even if the surface area changed, and this is supported by recent data. There is a gradient along the strobila of *H. diminuta* for the maximal velocity (V_{max}) of glucose absorption; the V_{max} for glucose uptake is five times greater at the anterior than at the posterior end (Pappas *et al.*, 1999). Similarly, the SAVR is 3.5 times greater at P100 than at P1300. The similarity of the values for glucose transport and the SAVR suggests that FAF does not change along the tapeworm's strobila. Thus, while the actual values reported herein for the surface areas and volumes of proglottids may not be accurate (for reasons discussed above), the relationship of the SAVR is probably representative of the changes that occur along the strobila of a living tapeworm.

A conspicuous feature of all flatworms, and the single feature for which the phylum Platyhelminthes is named, is dorso-ventrally flattening of the body. It has been suggested that in the free living flatworms, flattening is an adaptation to maximize the SAVR so that adequate amounts of oxygen can be absorbed. Based on the diffusion characteristics of oxygen through tissues, it has been estimated that a flatworm that absorbs oxygen through its dorsal and ventral surfaces can be no thicker than 1 mm (Alexander, 1979). Parasitic flatworms are, however, anaerobes, and some species are considerably thicker than 1 mm. Moreover, the data presented herein demonstrate that, while the SAVR is greater in the anterior-most proglottids of *H. diminuta* as compared to the posterior-most proglottids (14.6 versus 4.2), the 'flatness' of the tapeworm's proglottids does not maximize the SAVR. In fact, if the anterior-most proglottids of *H. diminuta* were the same length and volume but were cylindrical in shape, they would have a higher SAVR. This is somewhat paradoxical, considering that the anterior-most proglottids of *H. diminuta* are metabolically most active, at least in terms of glucose uptake and metabolism (Pappas *et al.*, 1999). Nevertheless, the data do support the hypothesis that the SAVR of rectangular, anterior proglottids is higher (more favourable) than in posterior proglottids.

As the proglottids of *H. diminuta* grow, they increase in size in all three dimensions, growth is allometric, and the data support the hypothesis that growth is highly predictable. Because the proglottids grow allometrically, at approximately P300 the surface area to volume ratios of the proglottids are the same regardless of their shape. Posterior to P300 the surface area to volume ratios are actually greater in rectangular than cylindrical proglottids, so 'flatness' is an advantage. This area (P300) is also the area of the tapeworm's strobila where organogenesis in beginning to occur (Pappas, 1998), and posterior to this section the reproductive organs are growing rapidly and eggs (oncospheres) are being produced. Perhaps these activities are more demanding metabolically than those activities occurring in the anterior part of the strobila, in which case allometric growth and flat, rectangular proglottids are advantageous since they maintain the most favourable SAVR. It would be interesting to know how the surface area to volume ratios change along the strobilae of other species of cestodes, especially those in which the proglottids are not only larger, but also shaped differently (i.e. longer than wide). Such data would be important in understanding the dynamics to tapeworm growth and testing hypotheses relative to the selective advantage of being 'flat'.

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