

A planimetric study of the mean epithelial thickness (MET) of the molluscan digestive gland over the tidal cycle and under environmental stress conditions

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ABSTRACT: Thinning of the digestive epithelium of marine molluscs may provide an index of environmental stress. Variability in epithelial thickness, as a consequence of the large variety of environmental and populational variables which could affect MET (Mean Epithelial Thickness), may counter the value of the index. Variation in MET of the intertidal gastropod *Littorina littorea* (L.) has been studied under experimental tidal conditions and, based on published data, under natural tidal conditions in the bivalves *Mercenaria mercenaria* (L.) and *Mytilus edulis* L. using a planimetric method. We found no significant variation in MET in all cases. The present results are discussed in relation to those obtained with *L. littorea* experimentally exposed to Cd and which show a significant reduction in MET after long sublethal exposure periods.

INTRODUCTION

Mean digestive epithelium thickness (MET) has been proposed as a histological index of environmental stress (Lowe et al., 1981; Tripp et al., 1984; Marigómez et al., 1986). The concept and its calculation are, however, not yet precisely standardized. This parameter is closely related to lysosomal labilization (Moore, 1986), which is accepted by WHO-FAO (GESAMP, 1980), as useful in environmental quality assessment. Both are based on the same biological feature: the induction of cellular lysis in the epithelium of the digestive gland of molluscs as a result of (a) detoxification processes, and (b) the general response to stress. As a consequence of enhanced secretion and cellular autolysis, the epithelium of the digestive diverticula becomes thinner, resulting in the formation of atrophic diverticula. These effects can be measured and expressed under the single concept of a reduction in MET, whose statistical significance can be tested. Lowe et al. (1981) showed the necessity of developing stress indices based on a simple methodology (i.e. MET, lysosomal latency) but, nonetheless, responsive to realistic environmental situations.

Several studies have described a thinning of the digestive epithelium in response to environmental stressors (Moore et al., 1978a, b; 1979; Lowe et al., 1981). The natural variability in MET associated with normal phasic digestive processes (Owen, 1972; Morton, 1973; Mathers, 1976; Robinson & Langton, 1980; Morton, 1983) has, however, rarely been, quantified. The morphometric measure of the relationship between digestive

cell height and tubule lumen width has been correlated with the percentage of digestive tubules in an absorptive stage (Robinson, 1983). These results indicate the validity of planimetry as an objective parameter of the morphological changes in the molluscan digestive gland. There are two events related to phasic digestion which could affect the value of MET of digestive diverticula over a tidal and/or digestive cycle.

The first of these deals with differences in the synchrony of the diverticula. Two types of digestive activity have been distinguished in molluscs: monophasic and diphasic digestion. In monophasic digestion, there is always a predominant tubule type which is dependent on the specific phase of digestion in which the digestive gland is involved at each moment of the tide, and which is also related to food availability. Monophasic digestion has been described by Morton (1956) for the bivalve *Lasaea rubra* (Montagu) from the high water zone, by Morton (1969; 1970 a, b, and c; 1971) for the bivalves *Dreissena polymorpha* Pall., *Cardium edule* (L.), *Macoma balthica* (L.), *Anodonta cygnea* L., and *Ostrea edulis* (L.), and by Morgan & Last (1982) for the freshwater prosobranch *Melanoides tuberculata* Müller. In these cases, the substitution of holding (type I) tubules by digestive (type II) tubules, and subsequent almost total substitution of type II by type III tubules has been observed over a tidal cycle. The diphasic pattern of digestion was first described (and the term coined) by McQuiston (1969). Owen (1972) reported upon this pattern for *C. edule* and *O. edulis*. In this case, the proportion of tubules type III remained almost constant over the tidal cycle. However, the type II tubules partially replace the type I resulting in an increase in epithelial thickness variability. MET would be slightly higher during this phase, just as the index developed by Robinson (1983) is when absorptive tubule numbers increase. These two tubule types are not dissimilar in thickness if compared with type III and IV tubules. Langton (1975) described a different pattern of diphasic digestion in *Mytilus edulis* L. In this case, the proportion of type I tubules was always lower than that of type II, and when digestion occurred the proportion of type II tubules increased. Thus, there is dominance of one tubule type without total replacement as in monophasic digestion, resulting in a small increase in MET and a noticeable reduction in epithelial thickness variability. In summary, we could define diphasic digestion as one in which there is alternation of the two simultaneously dominant types of tubules (I and II) (Langton, 1975) over the tidal cycle in relation to digestion.

The second digestive event that could reduce the significance of MET as a measure of environmental effects is whether or not the fragmentation stage is significant. Abundant fragmentation tubules have been associated with the final stages of monophasic digestion. Morton (1956) described monophasic digestion in *L. rubra* as including a dominant fragmentation stage. Owen (1972), however, rejected the pattern of Morton (1956) for *C. edule* and *O. edulis*, arguing a lack of complete fragmentation as described for *L. rubra* and *Scrobicularia plana* (da Costa), which also exhibits typical monophasic digestion (Owen, 1972). Langton (1975) attributed these apparently contradictory results to the time of exposure, underlining Owen's arguments to explain differences between different populations of the same species. These causes are: (a) correlation of feeding with external or internal rhythms; (b) the nature of the food, and (c) the mode of release of residual bodies. *L. littorea* does not show any evidence of a fragmentation stage such as that evidenced in *Mytilus* sp. (Merdsoy & Farley, 1973; Sáez, 1986).

Of the four types of diverticula morphology described in the digestive gland of

molluscs (holding or type I, absorptive or type II, fragmentation or type III, and regenerating or type IV) the most abundant are types I and II in examples of continuous (non-phasic) and diphasic digestion, which seems to be the most common. In such cases type III and IV tubules account for < 10 % of the total number of tubules. Such a feature has been described by Robinson & Langton (1980) and Robinson et al. (1981) for a subtidal population of *Mercenaria mercenaria* (L.), and by Langton (1975) for *M. edulis*.

Conversely, it is known that environmental stress induces the formation of atrophic epithelia (Couch, 1984). These are similar in thickness to type IV tubules resulting in a general reduction in MET of the tubule population. This reduction in MET might be used as an index to represent the proportion of atrophic diverticula induced by stressors. A problem might arise when studying animals with monophasic digestion in which tubules type III constitute an abundant subpopulation at some periods of the digestive cycle. This could occur in the case of *L. rubra* (Morton, 1956) and *O. edulis* (Robinson et al., 1981). Although there is a structural distinction between atrophy and normally thin tubules (Lowe et al., 1981; Couch, 1984), this may not be detected during routine analysis. It could be avoided after determination of the most appropriate sampling moments, or after selection of the most appropriate level on the shore in which the fragmentation stage is not present due to continuous food availability.

In the present work, variation in MET of the digestive gland of *Littorina littorea* (L.) under experimental tidal conditions has been investigated. The results are compared with those obtained when *L. littorea* is exposed to sublethal concentrations of cadmium leading to a stress response. It is a part of a larger project which includes the study of different environmental and populational variables which could affect the significance of MET in environmental stress assessment. The investigation deals basically with *L. littorea* because it may be considered an excellent indicator of pollution and a sentinel responsive to environmental stress. For comparison purposes, the non-planimetric data from other published works dealing with morphological variations in digestive tubules of several molluscs (Morton, 1956; Langton, 1975; Robinson & Langton, 1980; Robinson et al., 1981) have been transformed into planimetric ones and MET calculated.

MATERIALS AND METHODS

Experimental tidal conditions (4.5/7.5 h LT/HT and 12/12 h light/dark) were performed in the laboratory with the aid of a tide-simulator in which *Littorina littorea* individuals were acclimated for one week prior to removal. Unfed animals and *Fucus vesiculosus* L. fed animals were studied over two complete tidal cycles (24 h). Individuals were removed from the tide simulator every second hour and the posterior portion of their digestive gland dissected out and fixed in Bouin's liquid.

Paraffin-embedded 8- μ m serial sections were stained with haematoxylin-eosin (Pearse, 1976). Tubule-section shapes were drawn with the aid of a drawing tube attachment on a Nikon Optiphot microscope. The perimeters and the surfaces covered by both the outer and the inner shape of each tubule section were calculated by means of a Watanabe DT1000 digitizer connected to an Olivetti M24 personal computer. MET was estimated following the method based on the geometrical transformation of tubule-section shapes into a hypothetical trapezium (Fig. 1). The surface of this trapezium corresponds to the epithelium surface in the section (difference between the surface of

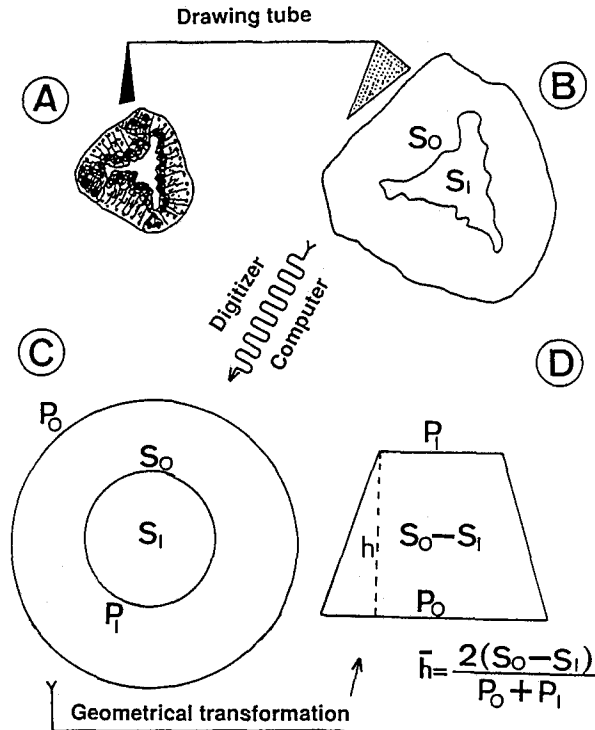


Fig. 1. Planimetric procedure used to measure the epithelial thickness of *Littorina littorea*. A: schematical representation of a tubule section observed under the light-microscope; B: magnified projection of the former tubule section (S_o = area covered by the outer shape, S_i = area covered by the inner shape); C: regular figure obtained from the magnified projection (the values S_o and S_i correspond to those of Figure 1B, P_o = perimeter of the new outer circumference, P_i = perimeter of the new inner circumference); D: hypothetical trapezium characterized by the same measures as in Figure C. The height of this trapezium (h) is considered to be the thickness of the tubule for this section. The mean h value for one animal is considered to be the MET value

both tubule shapes). The two bases of the trapezium have a fixed length: the perimeter of the circumference which contains a circle with the same surface as that limited by, respectively, the outer and the inner tubule shapes. Thus, from the equation $A = (B + b)/2 \cdot h$ (A , surface of the trapezium; B and b , major and minor bases; and h , height of the trapezium) the value h can be calculated. This value corresponds to the epithelial thickness of the tubule section. Five randomly selected tubule sections were measured in each of six paraffin sections (60–80 μm distant from each other) from two individuals of each sample. The mean value of the total statistical tubule population ($n = 60 = 5 \times 6 \times 2$) was considered to be the MET of each examined group.

The transformation of data from published works dealing with tubule variability in relation to tide and digestion was carried out in the following way. Usually these data are given as the average proportion (%) of each tubule type. Either from published photographs or scaled drawings, tubule-shape projections were measured in the digitizer following the procedure described above. The values calculated were used as a pattern

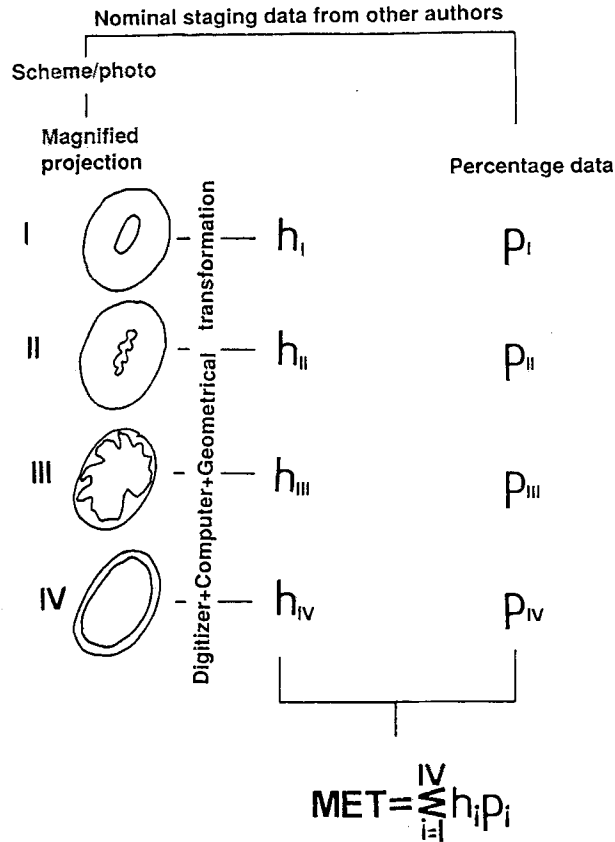


Fig. 2. Procedure used to transform the nominal-staging data from other authors into data comparable with MET values. h_i = h value for a pattern tubule-type calculated as described in Figure 1; p_i = percentage datum given by other authors to express the proportion of each tubule type. I, II, III, IV terms represent the four tubule types described in the digestive gland of molluscs

for each tubule type in each case. Thus, the MET of a sample was the sum of the products of each pattern of epithelial thickness by its corresponding percentage (Fig. 2). We are aware of the limitations of this procedure: it ignores the variability within and between tubule types (morphological change of digestive tubules is a continuous feature which is expressed in a complete set of intermediate figures). This would increase the variability of each sample and a more realistic measure of variability within each sample would be higher, and the confidence intervals wider than those calculated by means of the transformation procedure. Consequently, when two mean values are considered non-significantly dissimilar under these test conditions, the same conclusion should be obtained when considering a more realistic larger variability. The following experimental studies have been used as reference: Robinson & Langton (1980) for *Mercenaria mercenaria*, Morton (1971) for *Ostrea edulis*, and Langton (1975) for *Mytilus edulis*. Thus, the complete tidal cycles of *M. mercenaria* and *M. edulis*, as well as the extreme cases of digestive activity in these species and in *O. edulis* have been investigated.

On the other hand, we exposed individuals of *L. littorea* to Cd (1.25 mg/l) for 27 days. According to Marigómez et al. (1987), exposure to such a cadmium concentration should lead to a non-specific stress response because it is under the asymptotic LC50 value and results in a sublethal damage. Animals were maintained in filtered natural seawater for a 1-week period without food. Tanks were continuously aerated and water temperature ranged between 16 and 18 °C. Afterwards, cadmium was supplied as cadmium chloride for 27 days. Replicate control and cadmium exposure series were carried out. Two individuals were removed from each experimental group. Histological and analytical procedures were the same as described earlier. During the cadmium exposure period animals were fed on *Fucus vesiculosus*. Water and food were changed every second day.

One- and two-way ANOVAs (model I), the Student's t test for comparison between means, and the test of Student-Neumann-Keuls (S-N-K) have been the statistical procedures used to estimate the significance of the results (Sokal & Rohlf, 1979). The specific statistical treatment of each case is indicated in each group of results.

RESULTS

Figure 3 shows the results of the planimetric measurements of the digestive diverticula of *Littorina littorea* over two complete tidal cycles, under feeding and unfeeding conditions. ANOVA (Table 1) reveals no significant difference in the MET over a tidal cycle, or as a result of food digestion. Additionally, the S-N-K test showed no significant differences between the mean values.

Table 1. 2-way ANOVA table for the case of *L. littorea* subjected to experimental tidal conditions. FA: effect of food availability, SH: effect of the sampling hour, I: food availability \times sampling hour interaction, W: within groups

Source	Sums of squares	d.f.	Mean squares	F ratio
FA	4.12	1	4.12	0.24 (P > 0.1)
SH	334.91	12	27.91	1.60 (P > 0.05)
I	845.71	12	70.48	4.05 (P = 0.05)
W	452.73	26	17.41	
Total	1302.56	51		

The digestive diverticula of *Mercenaria mercenaria* (Fig. 4) show no significant variation in the MET due to tidal fluctuations (Table 2a). These results are in agreement with those obtained in the S-N-K test for comparison between mean values. ANOVA revealed a significant variation among epithelia in *Mytilus edulis* (Table 2b, Fig. 4). The S-N-K test indicated the existence of overlapped groups of epithelia in relation to MET, and only two well separated clusters could be distinguished from them. The first consists of 5 elements (6, 13, 15, 18 and 19 sampling hours) characterized by a higher MET value: 0.954 ± 0.015 arbitrary units. The second is constituted by the remaining 21 sampling hours (MET = 0.925 ± 0.016 arbitrary units).

Constancy in MET can be demonstrated even for the cases of extreme digestive stages. The peak intracellular digestive activity and the period in which intracellular

digestion is likely to be at a minimum were selected, and the morphology of the digestive diverticula studied in *M. mercenaria*, *M. edulis*, and *Ostrea edulis* (Robinson et al., 1981). In the present work these nominal-staging data were transformed into planimetric ones following the procedure formerly described (Fig. 2). The Student's t test allows us to conclude that the MET values at both extremes of digestive activity are not significantly different in any case (Table 3).

A strong effect of the treatment (Cd dosage and dosage-time interaction) on the populational variance in epithelial height has been recorded (Table 4). The mean value

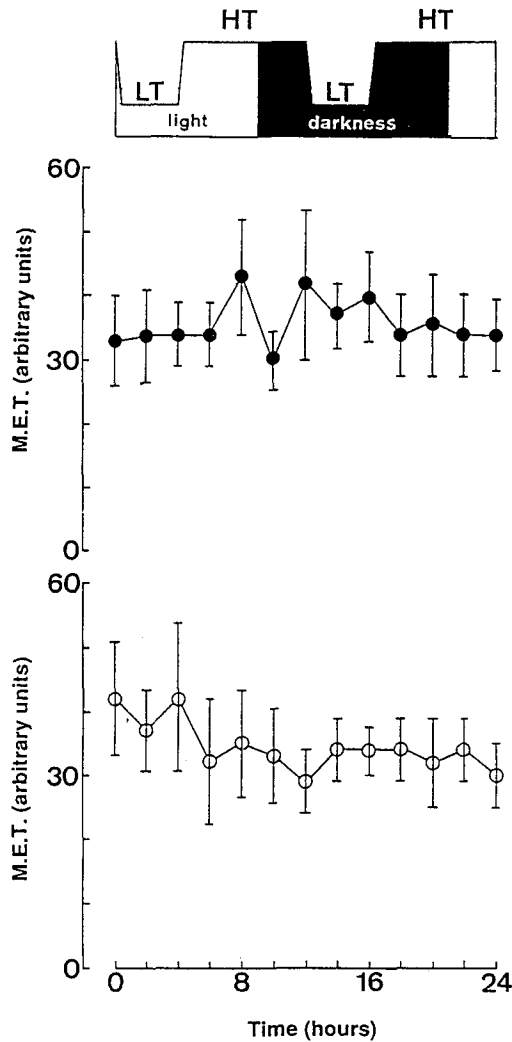


Fig. 3. Variations in the MET (arbitrary units) of the digestive diverticula of *Littorina littorea* over two tidal cycles (one complete light-dark period) under food availability (●) and food unavailability (○) conditions. Intervals represent the standard deviation for each case. HT = high tide, LT = low tide. n = 60 tubules for each point

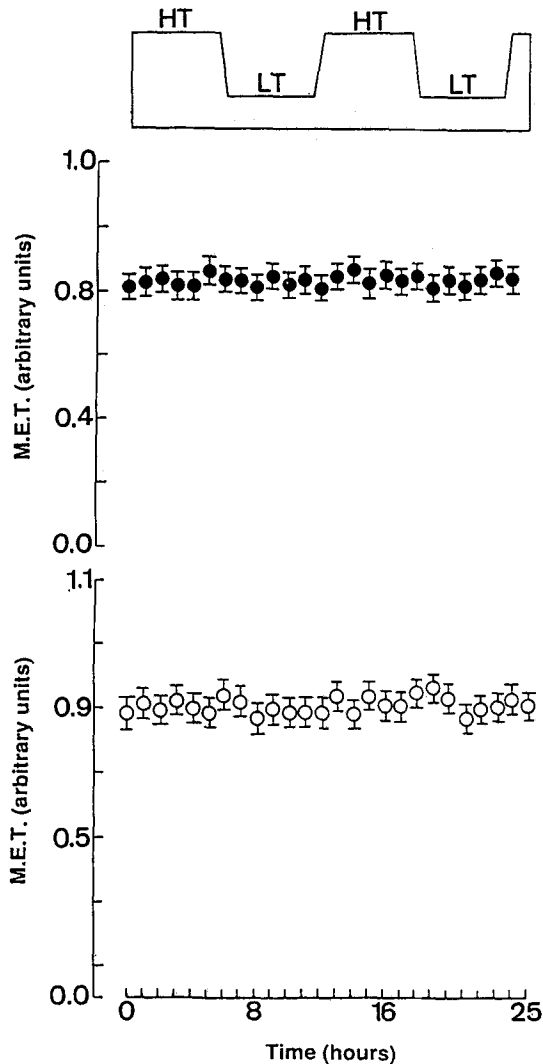


Fig. 4. Variations in the MET (arbitrary units) of the digestive diverticula of *Mercenaria mercenaria* (●) and *Mytilus edulis* (○) over two tidal cycles. Calculations from the studies of Langton (1975) and Robinson & Langton (1980), see text. HT = high tide, LT = low tide. Since results are based on percent data, $n = 100$ tubules for each point

decreased significantly (from 200 to 158 arbitrary units approximately). Figures 5C and D show the morphological changes in the stressed epithelia as compared with Figure 3, where the extreme cases of phasic digestion (high tide with food and low tide without food) are also illustrated.

DISCUSSION

The results obtained for *Littorina littorea* support our initial hypothesis of non-significant variation in the MET of its digestive gland due to tide or digestion (Fig. 3 and Table 1). This is in agreement with the lack of evidence of a fragmentation stage, as seen in *Mytilus* sp., (Merdsoy & Farley, 1973), as well as with the mero- and apocrine modes of release of residual bodies suggested by Sáez (1986) for *L. littorea*. The tidal regime in the

Table 2. 1-way ANOVA tables for *Mercenaria mercenaria* (a) and *Mytilus edulis* (b). AS: among samples (tide hours), WS: within samples (within animals)

Source	Sums of squares	d.f.	Mean squares	F ratio
<i>Mercenaria mercenaria</i>				
AS	0.57	24	0.024	1.19 (P > 0.1)
WS	49.48	2475	0.020	
Total (a)	50.05	2499		
<i>Mytilus edulis</i>				
AS	10.99	25	0.044	44.00 (P < 0.01)
WS	39.04	2574	0.010	
Total (b)	50.04	2599		

Table 3. Comparison between the MET (arbitrary units) of the extreme stages of the digestion for *Mercenaria mercenaria* (a), *O. edulis* (b), and *M. edulis* (c). $t_{0.05, (\infty)} = 1.96$. H: samples from the stage of high digestive activity, L: samples from low digestive activity, SD: standard deviations, n.s.: not significant

Species	H	SD	L	SD	ts
a	0.884	0.142	0.826	0.142	0.343 n.s.
b	0.838	0.201	0.676	0.162	1.090 n.s.
c	0.937	0.119	0.905	0.131	0.174 n.s.

Table 4. Results of the planimetric analysis of the digestive gland of *Littorina littorea* exposed to Cd for 27 days: 2-way ANOVA table. T: main effect of time, E: main effect of Cd-exposure, I: time \times exposure interaction, W: within groups of treatment

Source	Sums of squares	d.f.	Mean squares	F ratio
T	1867.91	1	1867.91	1.19 (P > 0.1)
E	100935.87	1	100935.87	64.21 (P < 0.01)
I (T \times E)	74351.20	1	74351.20	47.29 (P < 0.01)
W	371051.28	236	1572.09	
Total	548167.81	239		



laboratory corresponded to a mid-intertidal position on the shore in concordance with the zonation reported by Merdsoy & Farley (1973) for a natural population of *L. littorea*. According to these authors, mid-zonation could cause a lack of correlation between tidal cycle and phasic digestion. The digestion characteristics of this species seem to be associated with little variation in diverticular morphology. This is accurately expressed by a statistically constant MET, as expected.

Mercenaria mercenaria showed a similar behaviour in relation to MET variability (Fig. 4, Table 2a). The results could be justified on the basis of the description of the studied population (Robinson & Langton, 1980). According to these authors, all four tubule types are present in individuals in each sampling hour. It thus seems that intracellular digestion occurs continuously within the digestive diverticula, probably because of the subtidal zonation of this clam population. The former assertions are also in agreement with the results of Wilson & Latouche (1978) on a subtidal population of *Ostrea edulis*, i.e. the four tubule types were recorded every time. In both cases the proportion of tubules III and IV remains low and almost constant, while some changes in the tubule type I/type II ratio have been observed only as a result of different food availability: a high food-richness increases the proportion of type II tubules and vice versa. It is thus likely that alternation of the dominant tubule types does not significantly affect the MET in a subtidal population of *M. mercenaria* (Fig. 4, Table 2a).

M. edulis exhibits a pattern of diphasic digestion (Langton, 1977). The fragmentation stage is, however, sometimes evident. A similar peculiar pattern has also been reported in the scallop *Pecten maximus* L. (Mathers, 1976). In this case, almost 50 % of the tubule population shows type I morphology, while the other half are type II tubules. The fragmentation stage is abundant only at times in the digestive cycle. The present results on *M. edulis* are not in disagreement with the normal occurrence of a fragmentation stage. However, the total dominance of type I or II tubules results in a slightly higher MET value being reached only when absorptive tubule numbers increase over the 73 % of the total tubule population (Langton, 1975). The MET parameter seems to be a more accurate parameter than the MPTW (Mean Proportion of Tubule Width) (Robinson, 1983), because in those instances in which type III tubules are abundant (over 15 %) simultaneously with abundant absorptive tubules (11 and 23 hours) the MET value is low, while MPTW value is high (Robinson, 1983). These complementary results suggest the use of a combination of methods for studies of digestion. According to Langton (1975), 90 % of the tubules are either of type I or II, at mid-tidal levels, the following variables causing this: (a) the lack of feeding activity offers an equiproportional occurrence of both tubule types, and (b) aerial exposure and intermediate-term starvation increases the proportion of type I tubules. Therefore, normal changes in these tubule proportions might lead to slight but significant variations in MET which are unlikely to interfere with a stress-induced epithelial thickness reduction (Lowe et al., 1981).

Thus, no significant variation in MET could be detected for almost all the studied

Fig. 5. A: A paraffin section through the digestive diverticula of air-exposed unfed winkles; scale bar: 125 μm . B: Section from immersed fed winkles; scale bar: 75 μm . C: Section from control group before Cd-exposure; scale bar: 75 μm . D: Section from animals experimentally exposed to Cd (1.25 mg/l) for 27 days; scale bar: 75 μm . Differences are not observable between A, B and C photographs, whilst Cd-stressed digestive gland exhibits an advanced degenerative stage expressed in a thinner epithelium. (Stained with haematoxylin-eosin)

cases. Mid-tide molluscan populations, in which changes between holding and absorptive tubules are relevant, show a significant variation in MET values. Nevertheless, such variability does not seem to affect consistently the MET value for stress indication purposes because the most important variations occur between the type I and II tubule ratio. The extreme cases of *Lasaea rubra* and *Scrobicularia plana* (Morton, 1956; Owen, 1972), in which the fragmentation stage is predominant at some periods of the digestive cycle well correlated with the tide, should be studied to complete the field of possibilities in relation to the different types of digestion described in molluscs.

Although the digestive tubules of molluscs consist basically of the four mentioned types of tubule, it seems that stress conditions induce a replacement of the former by another tubule type that has been termed atrophic. Couch (1984) found that the occurrence of atrophic digestive epithelia is related to the presence of environmental irritants in the habitats of the oyster *Crassostrea virginica* (Gmelin). Lowe et al. (1981) described a reduction in height of the digestive cells beyond that demonstrated in a normal feeding cycle, in oil-exposed *M. edulis*. The normal synchrony of the digestive cells was lost to a point in which nearly all the tubules exhibited an appearance similar to that usually termed reconstituting (type IV). In fact, the reduction in the height of the digestive cell population would be an expression of the formation of a high proportion of atrophic diverticula.

The above phenomenon seems to be quite general, and has been reported to be a response to a great number of stressors. Thompson et al. (1974) describe a decrease in the digestive gland index, correlated to the proportion of each tubule type, as a consequence of starvation in *M. edulis*. Thompson et al. (1978) report upon a continuous intracellular digestion, where the four tubule types are always present, in *Mytilus californianus* Conrad from the lower shore. These authors describe a reduction in epithelial thickness caused by nutritional stress. Moore et al. (1978a and b; 1979) have observed the thinning of the digestive tubule epithelium, resulting from a loss of apical cytoplasm, in response to prolonged aerial exposure at elevated temperature, injection of 17β -oestradiol, and injection of anthracene in *M. edulis*. Tripp et al. (1984) also assert that some organic pollutants enhance reduction of the digestive cell height of *M. mercenaria*. Marigómez et al. (1986) conclude that copper exposure induces a reduction in digestive gland MET in the terrestrial gastropod *Arion ater* L. Finally, Axiak et al. (1988) have obtained similar results for the bivalve *Venus verrucosa* exposed to the water accommodated fraction of an oil. In view of these concordant results, it is therefore hypothesized that environmentally induced thinning of the digestive epithelium might be measured by means of MET.

Table 5. Comparison between the MET of the digestive diverticula of control and hypothetically stressed (80 % atrophic epithelia) *Mercenaria mercenaria* (a) and *Ostrea edulis* with high (b) and low (c) digestive activity. $t_{0.05}(\infty) = 1.96$. C: control populations, HS: hypothetically stressed populations, SD: standard deviations, s.: significant

Population	C	SD	HS	SD	ts
a	0.885	0.142	0.426	0.173	4.33 s.
b	0.838	0.201	0.490	0.185	2.97 s.
c	0.676	0.162	0.490	0.185	1.96 s.

Accordingly, the present statistical and graphical arguments suggest the validity of using MET for environmental stress assessment purposes in realistic situations. Thus, a significant reduction in MET beyond the lower values of this parameter recorded during digestion has been demonstrated in *L. littorea* exposed to sublethal concentrations of cadmium for 27 days.

The significance of such a reduction in MET even for the different types of phasic digestion is another question that should be considered in relation to the normal occurrence of abundant type III and IV tubules reported upon for some molluscan populations. The patterns of *M. mercenaria* (scarce occurrence of tubules type III and IV) and *O. edulis* (larger occurrence of type IV tubules) from the formerly described tubule populations (Table 5) have been the basis for these calculations. If a certain degree of environmental stress is supposed, a proportion of nearly 80 % atrophic diverticula might be reached (Couch, 1984). Similar conclusions have been formulated by Lowe et al. (1981), who do not refer to percentage values but indicate that nearly all the tubules show an appearance similar to type IV tubules. Thus, hypothetically stressed populations have been created by increasing the proportion of type IV tubules (up to 80 %). Previously, the present authors have found that the proportion of atrophic tubules and the MET value are linearly correlated (unpublished data). From the results shown in Table 5, a significant difference in MET, as well as a significant variance component due to treatment (hypothetical stress) can be concluded for all the cases, including that in which the fragmentation stage is abundant under normal conditions. However, these speculations require further experimental confirmation in field and laboratory studies based on the comparison between numerous molluscan species.

Acknowledgements. The authors wish to thank A. Mateo for his skilful technical assistance and photography-work, and J. Castellero for his excellent informatic support. The authors are specially indebted to Dr. D. M. Lowe and to some anonymous referees for their valuable criticism on the manuscript.

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