work that some of the phosphatidylethanolamine sites on the cytoplasmic side of the hippocampal membrane become methylated at the onset of LLP, so that a subsequent exposure of the membrane to labelled SAM would lead to a diminished incorporation of methyl groups into the membrane. On the other hand, the results may be explicable simply on the basis of a diminished activity of methylase I due to LLP. However, this explanation is less likely since SAM at 200 µM had no effect on phospholipid methylation and, therefore, the successive methylations catalyzed by methylases I and II could not have been suppressed.

Although the exact location is uncertain, there is adequate evidence[2,3,9,24] to indicate that the electrophysiological alterations found in LLP do not occur with stimulated axons or their target somata, but are confined largely to synaptic components. It is, therefore, reasonable to suggest that at least a part of this altered methylation occurs at the synaptic region. In this connection, it is pertinent to note the suggestion that an enhanced rate of phospholipid methylation could account for the effect of SAM in enhancing the rate of spontaneous firing of cerebral cortical neurons[23].

It has been pointed out that methylation of phosphatidylethanolamine and/or the rapid transit of the monomethylated lipid through the membrane decrease membrane viscosity[17]. It, therefore, appears possible that the enhanced membrane fluidity that occurs with phosphatidylethanolamine methylation may partially be responsible for potentiation. On the other hand, if a diminished methylase I activity occured, the resultant enhanced membrane viscosity could explain the decreased excitability of the presynaptic terminal after induction of LLP[9]. An alteration in membrane properties could also account for anatomical changes observed following tetanic stimulations of hippocampal inputs[25–27] which have been implicated in the enhancement of synaptic transmission[26–28].

In conclusion, it is suggested that LLP is associated with an altered membrane viscosity due to an altered rate of methylation of phosphatidylethanolamine. How this change relates to LLP development is at present unclear.

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Relationship between chlorophyll a content and protein content of invertebrate symbioses with algae or chloroplasts

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Summary. The chlorophyll a and protein contents of invertebrates containing autotrophic symbionts are indices of symbiont and host biomass, respectively, and the chlorophyll a content per unit protein is a good measure of symbiont: host ratio. For three different associations, the chlorophyll a content per unit protein is 0.01–0.02 µg µg^{-1}.
Key words. Symbiosis; invertebrate; algae; chloroplasts; chlorophyll a content; protein content.

The size of the population of autotrophic symbionts (unicellular algae or chloroplasts) in association with invertebrates is controlled within narrow limits[2,3]. This is probably achieved through the direct intervention of the host, by digestion or expulsion of excess symbionts or control of symbiont growth rate[2]. Basic to studies of the mechanism(s) by which the host may regulate its symbiont population is a reliable index of the relative size of the partners, i.e. of the 'symbiont:host ratio'. Two indices have been used: the ratio of the biomass of the separate partners and ratio of their volumes. The volume ratio can be estimated from quantitative studies of serial sections[4] or alternatively from the dimensions of the intact association and its symbionts if they approximate to regular shapes, such as spheres or cylinders[2]. Both methods to determine volume ratio are laborious and can be subject to bias and inaccuracy. To determine the biomass ratio, the partners are separated and the biomass of the isolated symbiont and host fractions measured. Although this approach is widely used, it is not satisfactory

because the symbiont fraction from most separation procedures is heavily contaminated with host material[5–7].

In this paper, a new measure of symbiont:host ratio is adopted, the ratio of chlorophyll a content to total protein content. It is further shown that chlorophyll a content per unit protein is 0.01–0.02 for three associations; namely, between the intertidal acoel flatworm *Convoluta roscoffensis* Graff and prasinophyte alga *Platymonas convolutae* Parke et Manton, between the sublittoral sacoglossan mollusc *Elysia viridis* Montagu and chloroplasts of the sea weed *Codium fragile* Pennant, and between the freshwater coelenterate 'green hydra' and algae of the genus *Chlorella*.

Materials and methods. Five strains of green hydra (see table) were maintained as described by McAnley[3]. Standard animals (i.e., individuals with one feeding bud) were assayed on the second day after feeding. *C. roscoffensis* were taken from the upper shore of five sandy beaches on Herm and Guernsey, Channel Islands listed in the table at the time of spring tide in May, 1980, and were assayed within three days. *E. viridis* were collected from the lower limit of the shore of Weymouth Bay, U.K., at the time of spring tide in March, 1983. They were maintained for 21 days prior to assay in aerated sea water at 15 °C under 12 h light/12 h dark regime at light intensity 20μ Einstein m^{-2} s^{-1}, and were provided with *Codium* plants.

The hydra were homogenized in ice-cold M solution, pH 7.6[8] (10 animals per ml) in a glass tissue grinder. Each individual of *E. viridis* was disrupted in 2.0 ml ice-cold A solution, pH 7.2[9] in a Teflon hand homogenizer. These procedures disrupted the animal cells but not the symbionts. The chlorophyll a content of the animals was determined spectrophotometrically. Homogenates of green hydra and *E. viridis* were centrifuged at 1000 × g for 5 min and the pellet of symbionts and contaminating host material was resuspended in 90% methanol. Pigment extraction was complete in 8 h at 18 °C, and the chlorophyll a content was determined by the equation of Holden[10]. The chlorophyll pigments of intact *C. roscoffensis* were extracted in 90% acetone at 18 °C for 15 min and the chlorophyll a content was estimated by the Parsons-Strickland trichromatic equation[11]. The protein content of the animals was determined by modified methods of Lowry et al.[12], with bovine serum albumin as standard. Details of the method of *C. roscoffensis* are given elsewhere[13] and the method of Peterson[14] was used for *E. viridis*. The protein of green hydra was solubilized by incubation of homogenates in 0.2 M NaOH for 3 h at 18 °C.

Results. The values of chlorophyll a and protein content per animal and chlorophyll a content per unit protein content for 5 strains of green hydra are shown in the table. Although there was considerable variation in the size of the different strains, as measured by protein content, the chlorophyll a content per unit protein was 0.010–0.014 for all strains. Comparable results were obtained for *C. roscoffensis*. The size of symbiotic animals under natural conditions varies widely, depending on the grain size of the sediment[15]. In this study, the mean protein content per animal ranged from 4 μg (Mouisonniere Beach) to 23 μg (Shell Beach); yet the chlorophyll a content per unit protein was 0.012–0.014 for animals from all sites (see table). *E. viridis* is much larger than green hydra and *C. roscoffensis*, and so 10 individual animals of differing sizes were analyzed. Their protein content lay in the range 0.50–1.98 mg. The values of chlorophyll a per unit protein varied between 0.014 and 0.020 (see table), with no consistent differences between individuals of high and low protein content.

Discussion. The total protein and chlorophyll a contents of the associations can be considered as indices of host and symbiont biomass, respectively. The total protein content approximates to host protein because the host comprises more than 90% of total protein in those associations which have been investigated, e.g., green hydra[5], corals[16], *C. roscoffensis*[17]. The amount of photosynthetic pigments is correlated with other indices of biomass in unicellular algae (although there is some variation with environmental conditions)[18, 19]. An example of particular relevance to the present study is that the chlorophyll a content per cell of *Chlorella* symbionts of green hydra is significantly correlated with algal cell volume (calculated from data from Douglas and Smith[20]). Chlorophyll a was chosen in this study because it is the major photosynthetic pigment of all algae[19] and so results obtained with associations containing symbionts with different accessory pigments can be compared.

The parameter chlorophyll a per unit protein is potentially a very useful index of symbiont:host ratio. It has several advantages over alternative measures of symbiont:host ratio. Rapid and reliable assays which require only small amounts of tissue are available for both chlorophyll a and protein content, and this index is not dependant on quantitative separation of symbiont and host, for which no satisfactory methods have been developed for most associations.

The three associations examined here were obtained from different habitats, were maintained at different light intensities and involve very different host and symbiont taxa and a wide range of host size, both within and between the different associations. Despite this, they had very similar values of chlorophyll a per unit protein. Other workers[21] have obtained comparable values for the symbiosis between the anthozoan *Aiptasia diaphana* and dinoflagellate *Symbodinium microadriaticum*, although, in contrast to this study, they note some variation between animals of different sizes; see table. It is unlikely that the constancy of this parameter between these diverse associations is fortuitous. It may represent a particularly favorable level of the symbiont population for the associations; in which case the same level is favorable for the different associations. At first sight, this is surprising because the nutritional contribution of the autotrophic symbionts to the different associations varies widely. For example, whereas *C. roscoffensis* is almost entirely dependent on its symbionts for nutrition[22], green hydra requires regular feeding[23]. However, recent studies of the green hydra association[20] suggest that non-nutritional factors are important in determining the level of the symbionts. One possible

Protein content, chlorophyll a content and value of chlorophyll a per unit protein for green hydra, *C. roscoffensis* and *E. viridis*

Organism	Chlorophyll a (μg) per animal	Protein (μg) per animal	$\frac{\text{Chlorophyll a}}{\text{protein}}$
Hydra – strain			
Florida	0.0583 ± 0.0025	5.43 ± 0.26	0.0107 ± 0.0010
Frome	0.1087 ± 0.0003	7.67 ± 0.18	0.0142 ± 0.0004
Jubilee	0.1301 ± 0.0005	11.07 ± 0.47	0.0118 ± 0.0005
European	0.1415 ± 0.0006	11.23 ± 0.23	0.0126 ± 0.0003
Bracken Hill	0.1569 ± 0.0002	11.60 ± 0.42	0.0135 ± 0.0005
C. roscoffensis – site of origin			
Mouisonniere Beach	0.057 ± 0.007	4.7 ± 0.5	0.0123 ± 0.0028
Pequerie Bay	0.107 ± 0.012	8.8 ± 0.4	0.0122 ± 0.0019
Port es Vallais	0.177 ± 0.009	12.9 ± 0.3	0.0137 ± 0.0010
Rocquaine Bay	0.183 ± 0.019	14.2 ± 0.9	0.0129 ± 0.0022
Shell Beach	0.313 ± 0.007	22.9 ± 0.4	0.0136 ± 0.0005
E. viridis	19.54 ± 2.64	1216 ± 147	0.0161 ± 0.0041
Aiptasia diaphana	–	15,000–30,000	0.0055–0.0070
	–	4,000–10,000	0.0080–0.0140

Protein and chlorophyll a contents of triplicate samples of hydra and *C. roscoffensis* and of 10 individuals of *E. viridis* were assayed, and values of mean ± SE are shown. SE of $\frac{\text{chlorophyll a}}{\text{protein}}$ is:

$$\left(\text{mean of } \frac{\text{chlorophyll a}}{\text{protein}}\right) \times \left(\frac{\text{SE}}{\text{mean}} \text{ of protein} + \frac{\text{SE}}{\text{mean}} \text{ of chlorophyll a}\right).$$

The values of $\frac{\text{chlorophyll a}}{\text{protein}}$ of *A. diaphana* of different protein contents are obtained from Svoboda and Porrman[21].

approach to identify such factors would be to characterize the experimental conditions which perturb the symbiont:host ratio.

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A simple method for the preparation of hydra chromosome spreads: introducing chromosome counts into hydra taxonomy[1]

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Summary. A simple method to prepare chromosome spreads of hydra is described. Chromosome counts for a non-symbiotic 'brown hydra', *Hydra vulgaris attenuata,* and the Swiss strain of a symbiotic 'green hydra' indicated a diploid number of about 2n = 30 in each case. It is suggested that chromosome number may be used to define hydra species more precisely.
Key words. Hydra; chromosomes; taxonomy.

The small freshwater coelenterate *Hydra* has been used for many years in research on animal behavior, cell biology, morphogenesis and symbiosis[2].

Hydra are commonly divided into two groups: non-symbiotic 'brown hydra' and hydra containing symbiotic green algae ('green hydra'). Morphological characteristics, e.g. size, and structure of nematocytes and embryotheca have been used to distinguish between different species within each group[3,4]. However, these criteria are not entirely satisfactory (e.g. because of variation with environmental conditions) and as a consequence of this taxonomic ill-definition, published data are frequently difficult to interpret.

In the following communication we describe a quick and simple method to prepare hydra chromosomes for counts, so that hydra can be defined more precisely.

Our chromosome counts are consistent with previous results for a brown hydra, *H. vulgaris*[5,6], but differ from published chromosome numbers of a green hydra, *H. viridis*[7].

Materials and methods. Organisms. A brown hydra identified by R.C. Campbell as *H. vulgaris attenuata* and the Swiss strain of green hydra were used in our studies. Stock cultures were grown in M solution[8], but without TRIS, at 15 °C in a 12/12 h light/dark regime. The hydra were fed three times a week with freshly hatched nauplii of *Artemia* sp.

Procedure. For chromosome analysis, hydra were fed and placed for 18 h in growth medium containing 0.1–5.0 μg colcemid ml^{-1} (*H. vulgaris*) or 0.2 μg colcemid ml^{-1} (green hydra). (There was no indication that colcemid concentration influenced the results over the range tested.) The hydra were then rinsed, cut into several small pieces and incubated in distilled water. After about 20 min the hydra pieces were fixed in 3:1 methanol:acetic acid for 3–5 min. Hydra may be left in this fixative, in a refrigerator, for several days.

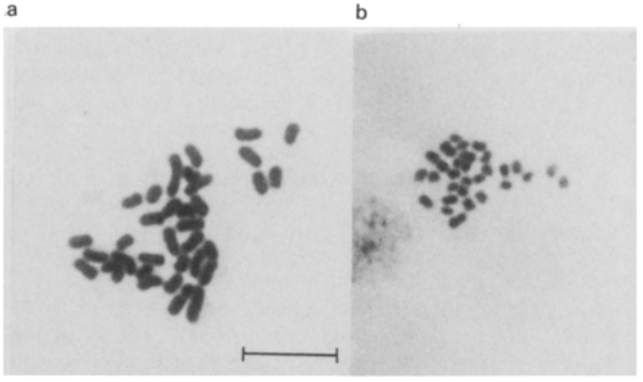

Figure 1. Representative photomicrographs of metaphase spreads of *H. vulgaris* (a) and green hydra cells (b). The chromosome counts for these cells were 33–34 and 30–32, respectively. Note that in our preparation the total chromosome area tends to be smaller in preparations of green hydra cells. Scale = 10 μm.