females, and 69 ± 7.38 and 55 ± 2.17 in males. Gonadectomy decreased the number of larvae from 82 ± 4.33 to 65 ± 6.04 and 73 ± 4.14 to 57 ± 5.50 in females, and from 69 ± 7.38 to 46 ± 3.27 and 55 ± 2.17 to 46 ± 1.83 in males. All differences were statistically significant. Simultaneously gonadectomy significantly decreased the number of nonbudding larvae and increased the number of budding larvae in both male and female mice.

The mean number of nonbudding larvae decreased from 67 ± 3.80 to 45 ± 5.76 and from 61 ± 3.69 to 41 ± 5.58 in gonadectomized females and from 56 ± 6.52 to 24 ± 3.09 and 44 ± 2.48 to 26 ± 1.43 in gonadectomized males in experiments 1 and 2 respectively.

The mean number of budding larvae increased from 15 ± 1.29 to 21 ± 1.86 and 13 ± 1.04 to 16 ± 1.11 in gonadectomized females, and from 12 ± 1.91 to 22 ± 1.40 and 11 ± 1.05 to 21 ± 1.09 in gonadectomized males. The number of buds on each of the budding larvae ranged from 1 to 4 in control mice and from 1 to 10 in gonadectomized mice.

The larvae from gonadectomized mice were larger than those from controls. The larvae from gonadectomized males measured on the average 3.2 × 1.8 mm, those from control males 2.3 × 1.4 mm. The larvae from gonadectomized females measured 3.3 × 1.6 mm and those from control females 2.8 × 1.5 mm.

Thus the present experiments showed that gonadectomy of mice slowed down considerably the asexual reproduction of *Taenia crassiceps* cysticerci and increased the average size of the larvae. The results were thus similar to those obtained with *Mesocestoides* tetrathyridia in intact and gonadectomized mice[1].

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Assimilation and Retention of Tocopherol and Chlorophylls in the Rotifers *Brachionus calyciflorus* and *Asplanchna sieboldi*

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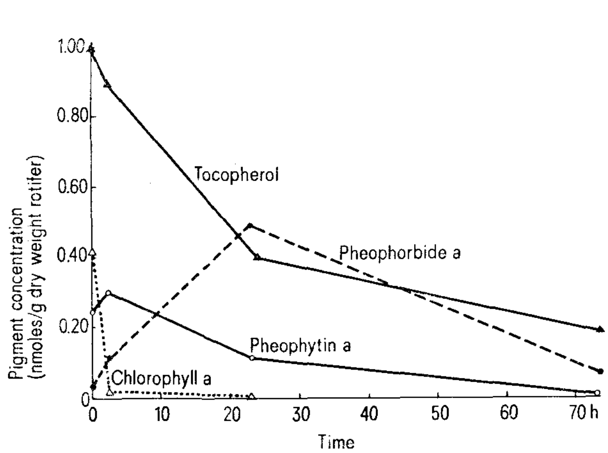
Summary. Tocopherol (vitamin E) is selectively assimilated by the rotifers *A. sieboldi* and *B. calyciflorus* as compared to chlorophyll pigments, and is retained in these rotifers for longer periods than the chlorophyll pigments. While tocopherol is lost at the rate of only about 50% per 24 h period, chlorophyll a and b are converted to pheophytin a and b and finally pheophorbide a and b in 24 h and then rapidly lost.

Dietary tocopherol (vitamin E), which is synthesized uniquely by plants, plays a major role in regulating nongenetic polymorphism in the predatory rotifer *Asplanchna sieboldi*[2,3]. The importance of tocopherol to *Asplanchna* was suggested by its retention, in vivo stability, and efficient transfer between parthenogenetic generations[4,5]. The purpose of the present study was to determine the ability of *Asplanchna* and one of the prey organisms to assimilate and retain tocopherol in relation to other compounds synthesized uniquely[6] by plants. Accordingly, we undertook a quantitative study of the concentrations of chlorophyll pigments a and b, including their degradation products, and tocopherol in the tissues of both *A. sieboldi* and *Brachionus calyciflorus* which had been feeding on the alga *Euglena gracilis*. In this way we could obtain comparative information on the degree to which these compounds were assimilated and retained.



Concentration of chlorophyll a, chlorophyll a products and tocopherol with time in *Brachionus calyciflorus* (run 1) following a several day exposure to a chlorophyll- and tocopherol-containing diet.

Materials and methods. The rotifer *Asplanchna sieboldi*, clone 12Cl, was cultivated on a chlorophyll- and tocopherol-free diet of *Paramecium aurelia* as described by Gilbert[5] in glass evaporating dishes containing about 200 ml of media. For several days prior to the experiment the diet was made to include chlorophyll pigments and tocopherol by adding a suspension of sonicated *Euglena gracilis*, strain Z, grown axenically as in Gilbert[3]. After initiation of the experiment the diet was changed back to *Paramecium* alone. The rotifer *Brachionus calyciflorus* was cultured on the chlorophyll- and tocopherol-free yeast *Rhodotorula glutinis* as in Litton and Gilbert[7]. Several days before the experiments started these rotifers were cultured on *Euglena gracilis*, strain Z, as described by Gilbert[2]. After the experiments started, the diet was changed back to yeast.

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The basic design of the experiments was as follows. Rotifers which had been feeding on algal material for several days were washed free of uningested algae or algal fragments using inorganic rotifer medium and nylon screening. Samples of rotifers were taken for analysis, while the remainder of them were transferred back to the chlorophyll- and tocopherol-free diets for sampling at subsequent periods. Samples of *Euglena* and *Rhodotorula*, as well as both rotifers which had been cultured for long periods of time on chlorophyll- and tocopherol-free diets, were also taken for analysis.

Samples for pigment analysis were homogenized[3,8]. After extraction in acetone in the dark at 4 °C a small aliquot of the homogenate was removed prior to extraction for dry weight measurements according to procedures in LITTON and GILBERT[7]. Estimation and differentiation of chlorophyll a and b, chlorophyllide a and b, pheophytin a and b and pheophorbide a and b were completed using a series of sensitive fluorometric assays and acidic reactions described by WHITE et al.[9]. A Turner Model 430 Spectrofluorometer was used throughout the study. Pure samples of chlorophyll a and b (Sigma Chemical, St. Louis, Missouri, USA) served both as standards and as materials for the preparation of standards of chlorophyll degradation products according to methods cited in WHITE et al.[9]. An aliquot of the homogenate was also lyophilized and extracted with ethanol for the spectrofluorometric assay for α-tocopherol as described in LITTON and GILBERT[7].

Results and discussion. Results of two experiments with *B. calyciflorus* and one with *A. sieboldi* are presented in the table. This table shows the change in concentration of chlorophyll (and some of the degradation products) and tocopherol in µg/g dry weight rotifer. The figure presents the same data on a molar basis for one of the *Brachionus* experiments.

The results showed that tocopherol was removed from the *Euglena* food source by both *B. calyciflorus* and *A. sieboldi* and retained within their bodies. Since tocopherol is efficiently transferred from mother to offspring in *Asplanchna*[4] the observed decreases in tocopherol concentration per g dry weight rotifer by about 50% in 24 h may be due to this selective transfer. A similar decrease was also noted in the 24–72 h period for *Brachionus*.

Chlorophyll a and b concentrations decreased rapidly in the first 3 h after ingestion of plant material in both *B. calyciflorus* and *A. sieboldi*. In the repeated experiment for *B. calyciflorus*, which had even less suspended algal material in the washed culture, chlorophyll a and b concentrations were zero when measured 3 h after *E. gracilis* ingestion. Chlorophyllide a was detected in very small

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Concentration of chlorophyll, chlorophyll degradation products and tocopherol with time in *Brachionus calyciflorus*, *Asplanchna sieboldi*, *Euglena gracilis and Rhodotorula glutinis*

Time (h) after removal from *E. gracilis* food source and culture on a chlorophyll- and tocopherol-free diet	Pigment concentration (µg/g dry weight organism)[a]						Tocopherol concentration[a] (µg/g dry weight organism)
	Chlorophyll a	Chlorophyll b	Pheophytin a	Pheophytin b	Pheophorbide a	Pheophorbide b	
Brachionus calyciflorus							
Run 1							
0	0.370	0.021	0.210	0.019	0.016	0.008	0.43
2–3	0.096	0.011	0.265	0.003	0.167	0.013	0.39
24	0.038	0	0.095	0	0.296	0.031	0.19
72	0	0	0.003	0	0.043	0	0.09
Control (no *E. gracilis in diet*)	0	0	0	0	0	0	0
Run 2							
0	0.260	0.009	0.010	0.003	0.005	0	0.31
2–3	0	0	0.018	0	0.089	0	0.28
24	0	0	0	0	0.101	0	0.13
72	0	0	0	0	0.016	0	0.06
Control (no *E. gracilis* in diet)	0	0	0	0	0	0	0
Asplanchna sieboldi							
0	0.490	0.014	0.356	0.085	0.003	0	0.93
20.5	0.016	0	0.097	0	0.385	0.010	0.37
Control (no *E. gracilis* in diet)	0	0	0	0	0	0	0
Euglena gracilis	68,320.0	12,260.0	0	0	0	0	794.0
Rhodotorula glutinis	0	0	0	0	0	0	0

[a] 0 is < 0.01 µg for all chlorophyll and degradation products determinations and < 0.005 µg for tocopherol determinations.

concentrations (0.003 µg/g dry weight rotifer) in *B. calyciflorus* (run 1) following ingestion of plant material, but at no other time in this or subsequent runs of *B. calyciflorus* or *A. sieboldi*. Chlorophyllide b was not detected in any experiments. Chlorophyllides are degradation products formed by the action of the plant enzyme chlorophyllase and therefore probably do not represent a major degradation pathway in zooplankton. Pheophytin a and b concentrations in *B. calyciflorus* were greatest at the 3 h sampling point, decreased rapidly after 24 h, and were present in negligible or non-detectable amounts after 72 h. These observed chlorophyll degradation pigments are known to be produced in the digestive tract of other zooplankton rapidly after ingestion of viable chlorophyll[10, 11]. In *A. sieboldi* pheophorbides a and b were still at high levels after 20.5 h.

The curves presented in the figure for the second experiment with *B. calyciflorus* show that chlorophyll a and its degradation products are conserved in the rotifer body during the first 24 h and subsequently decrease with time. After 24 h the sum of chlorophyll a + pheophytin a + pheophorbide a concentrations was 94.7% of the chlorophyll a and pheophytin a concentration at the beginning of the experiment. At 72 h the degradation products represent only 11% of the initial chlorophy l a + pheophytin a present initially. A similar pattern may account for chlorophyll b but it is occasionally undetected owing to the very small concentrations of chlorophyll b and pheophytin b relative to chlorophyll a. The high concentrations of pheophytin a or b relative to chlorophyll a or b present at the beginning of an experiment is probably due to rapid degradation of the chlorophyll molecule upon contact with the probably acidic rotifer gut.

The retention of chlorophyll degradation products for up to 72 h, following transfer to a chlorophyll-free diet, suggests uptake of chlorophyll a and b or their degradation products. Evidence for the uptake of chlorophyll in rotifers[12, 13] and other invertebrates[14, 15], and its possible subsequent use in pigmentation[16] and biosynthesis is rare but not unknown.

The most important information in the present study concerns the relative uptake and retention of tocopherol as compared with chlorophyll. The measured molecular tocopherol : chlorophyll a ratio in *E. gracilis* (1:41.6) indicates the low concentration of tocopherol relative to chlorophyll a molecules; the tocopherol : chlorophyll b ratio is 1:7.3. Both measurements on *B. calyciflorus* feeding on *Euglena* show high molecular tocopherol : chlorophyll a ratios (2.4:1 and 2.5:1) and tocopherol : chlorophyll b ratios (43.5:1 and 72.0:1). In *A. sieboldi* the initial molecular tocopherol : chlorophyll a ratio was 4.4:1 and the tocopherol : chlorophyll b value was 154.3:1. The small discrepancy in tocopherol : chlorophyll a or b ratios between *A. sieboldi* and *B. calyciflorus* may be due to their different modes of feeding. *Brachionus* is a herbivore and ingested algae and algal fragments directly whereas *Asplanchna* is a carnivore and obtained algal products secondarily by feeding on *Paramecium*. The *Paramecium* diet of *A. sieboldi* probably contained a higher tocopherol : chlorophyll pigment content than the algae eaten directly by *B. calyciflorus* owing to minimal tocopherol degradation and high chlorophyll pigment degradation and loss during intracellular digestion. Clearly then, the rotifers are assimilating tocopherol in preference to the more abundant chlorophyll a and b molecules.

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The Relationship of the Vertical Distribution of Japanese Conifers to the Maltol Contents in their Leaves

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Summary. Of 35 species of coniferous trees in Japan, only 4 species, found at subalpine zone, contain maltol in their leaves. Their leaves are also characteristic for their high content of sugar which protects the plants against freezing. It is interesting from the viewpoint of plant ecology that only the above 4 species contain high amounts of sugars and maltol in their leaves because maltol is biosynthesized from sugars.

There are 35 species of coniferous trees in Japan, a relatively large number considering Japan's geographical position. Of these 35 species, only 4 contain maltol (2-methyl-3-hydroxypyran-4-one) in their leaves. They are *Abies mariesii* (Japanese name, Oushirabiso), *A. veitchii* (Shirabiso), *A. homolepis* (Urajiromomi) and *Tsuga diversifolia* (Kometsuga). It is characteristic of these 4 species that they are found only at elevations between 1500 and 2900 m, that is to say that they are common to the subalpine zone alone and are not found lower (Figure 1). Japan's other 31 coniferous species (19 species of the Pinaceae family, 2 species of the Taxodiaceae family, and 10 species of the Cupressaceae family) are generally found at lower levels, ranging from sea level to 2000 m. In the lower subalpine zone (1500–2000 m), the vertical distribution of the above-mentioned 4 conifers and the rest of Japan's coniferous species overlap. It should be noted, however, that while *A. mariesii* and *T. diversifolia* resemble *Abies firma* (Momi) and *Tsuga sieboldii* (Tsuga) respectively, only the former leaves bear rich maltol (0.5–3.4%). The vertical distribution of *Pinus pumila* (Haimatsu) is wide (Figure 2). In Honshu it can be found at elevations as high as 3200 m, and in Hokkaido it is common from sea level to the critical forest limit, which, owing to the latitude, is at about 2300 m. Maltol is not found from the leaves of *P. pumila*. Maltol was first isolated from *Larix decidua*[1], a species common to Europe. It is interesting to note that maltol is not found in *L. leptolepis*, the Japanese variety of *L. decidua*, which is found below the subalpine zone.