our inability to detect melatonin was probably not due to its being produced and then rapidly degraded in the pineal. It thus seemed that the melatonin deficiency in the pineal glands of C57BL mice might be due to defects in one or both of the enzymatic steps in the synthesis of melatonin from serotonin.

Because two to four pineal glands must be pooled to make reliable enzyme measurements, we were not able to examine directly the segregation pattern of enzyme activities in F_2 animals. The activities of the two enzymes (NAT and HIOMT) involved in the synthesis of melatonin from serotonin at hour 22 in C57BL mice, FDS mice, and F_1 hybrids are shown in Fig. 3. The segregation experiments, in which melatonin was measured, suggested that two genes were involved in melatonin deficiency. Our measurements of NAT and HIOMT enzyme activity suggest that mutant alleles of these two genes are responsible for NAT deficiency and HIOMT deficiency. The observed intermediate enzyme levels in the F_1 hybrids may reflect heterozygosity at both loci.

We hypothesize that the melatonin deficiency observed in C57BL mice is due to the presence of recessive mutant alleles at two independently segregating autosomal loci, one of which controls NAT activity and the other HIOMT activity. One additional observation supports this hypothesis. Although pineal glands of NZB mice do not contain melatonin, we have been able to measure NAT activity (241.1 ± 21.8 pmole per gland per hour, $n = 7$) and the presence of its product *N*-acetylserotonin (by HPLC-EC) in their pineal glands at hour 22. We were unable to detect HIOMT activity. This suggests that the two enzymes are independently regulated and that there may exist three distinct forms of melatonin deficiency involving these loci: (i) the genes for both NAT and HIOMT may be defective, as we suspect in C57BL mice; (ii) only the gene for HIOMT may be defective, as we suspect in NZB mice; and (iii) only the gene for NAT may be defective (no example yet found).

Melatonin can act as an antigonadal factor regulating reproductive responses of some mammals (*3*). Its role, if any, in regulating reproduction in the mouse is unknown. Domesticated mice have been selected to breed in unusual, laboratory environments, and vigorous inbreeding is known to reduce fecundity. Under such conditions the presence of melatonin might exert a negative effect on reproductive success. If that were so, pineal melatonin synthesis might well have been inadvertently eliminated in the course of selection for inbred strains that breed well in the laboratory.

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4. Mice (50 to 120 days of age) were decapitated at various times of the day or night; the skull caps with attached pineal glands were then cut away and immediately placed on dry ice. Samples were frozen (−20°C) and used within 30 days for melatonin assay or within 4 days for enzyme assays. An infrared image converter (FJW Industries) was used for all manipulations in darkness. Melatonin was extracted and measured by radioimmunoassay in a modification of the method of M. Rollag and G. Niswender [*Endocrinology* **98**, 482 (1976)], and NAT and HIOMT activity were measured by a modification of the method of D. Sugden *et al.* [in *Methods in Biogenic Amine Research*, S. Parvez *et al.*, Eds. (Elsevier, New York, 1983), p. 567]. Mice were housed on wood shavings in plastic cages with unlimited food and water available under a 12:12 light-dark regime. The room temperature was kept at 21° ± 1°C. C57BL/6J mice were bred in our colony, BALB/c were obtained from J. Weston at the University of Oregon, and wild-derived inbred strains were obtained from E. M. Eicher, Jackson Laboratory, Bar Harbor, ME. FDS mice were shipped from the laboratory colony of F. H. Bronson at the University of Texas, and other mice were purchased from the Jackson Laboratory.
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8. Even though melatonin synthesis might be completely blocked by the absence of either enzyme, activity of the other enzyme could result in the synthesis of indoles other than melatonin which cross-react with the melatonin antibody. For example, in $F_1 \times C57BL$ matings, two-thirds of those progeny which, according to our hypothesis, should not be able to synthesize melatonin, bear a dominant allele for either NAT or HIOMT; in $F_1 \times F_1$ matings six-sevenths of the mice not able to synthesize melatonin bear such an allele and therefore might produce cross-reacting indoles. It may also be that all mice have low levels of NAT (see NAT levels for C57BL mice in Fig. 3) and that, when HIOMT is present, low levels of authentic melatonin are made.

9. We thank G. Cahill and J. Postlethwait for helpful comments on the preliminary manuscript, E. M. Eicher for supplying wild-derived inbred strains, and F. H. Bronson for his gift of the FDS mice and for valuable discussion. This work was supported by NIH grant 13162 and NSF grant DCB-8409010 to M.M. and by grants from the Fogarty International Fellowship (F05TW03377) and Medical Research Foundation of Oregon to S.E.

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Bacterial Grazing by Planktonic Lake Algae

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Six common species of lake algae were found to ingest bacteria. The ingestion rates measured were of the same magnitude as those recorded for marine microflagellates totally dependent on external sources of carbon. A large biomass of *Dinobryon* species removed more bacteria from the water column of a lake than crustaceans, rotifers, and ciliates combined.

THE VIEW THAT PHYTOPLANKTON receive all their energy through photosynthesis was first placed in doubt when it was shown that some algae supplement their carbon supply by taking up dissolved organic carbon (*1*). The phytoplankton could no longer, therefore, be viewed as a strictly autotrophic community, even though this uptake is normally modest and provides only a small fraction of the total carbon acquired (*1*). We now provide evidence that at least some natural phytoplankton are phagotrophic and apparently obtain a substantial fraction of their energy and nutrients by ingesting bacteria at rates very similar to those measured for nonphotosynthetic microflagellates.

The study was carried out in Lac Cromwell, Quebec, on 7 to 8 July 1984. Tracer quantities of bacteria-sized fluorescent latex beads (diameter, 0.6 μm), were released into the plankton caught in a Haney in situ grazing chamber (*2*) at a depth of 3 m. After 1, 4, 7, 10, 13, or 17 minutes the chamber was retrieved and the plankton were preserved and stained (*3*). Sample aliquots were poured onto Nuclepore filters (pore size, 10 μm) for epifluorescence counting of beads ingested by the plankton. We confirmed that the bead uptake rate was representative of bacterial uptake by performing experiments in which algae were exposed to mixtures of beads and tritium-labeled bacteria (*4, 5*).

Four species of the common planktonic alga *Dinobryon* were major consumers of bacteria in Lac Cromwell: *D. sertularia*, *D. sociale* v. *americanum*, *D. cylindricum* (Fig. 1a), and *D. bavaricum*. Other members of the Chrysophyceae, *Uroglena americana* (Fig. 1b) and *Uroglena conradii*, also ingested particles. The "grazing" algal community was found to be most concentrated in a thin layer within the thermocline; there *Dino-*

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Table 1. Results of feeding experiments in Quinn Bay, Lake Memphremagog. Bacterial abundance was 5 million cells per milliliter. Clearance rate is the volume of water from which bacteria were removed per day. Results comparable to these can be derived from many lakes, since *Dinobryon* is a common dominant alga (*11*). *Dinobryon* abundance in eastern U.S. lakes averages 142,000 cells per liter when present and 633,000 cells per liter when it is dominant (*12*).

Organism	Mean abundance (organisms per liter)	Individual mean clearance rate (ml/day)	Group mean clearance rate per liter ±95 percent confidence limit (ml/day)
Crustaceans	19.4	0.22	4.3 ± 2.1
Rotifers	238	0.014	3.3 ± 1.3
Ciliates	15,000	0.0010	15.6 ± 11.0
Dinobryon	479,000	0.00014	69.1 ± 19.8

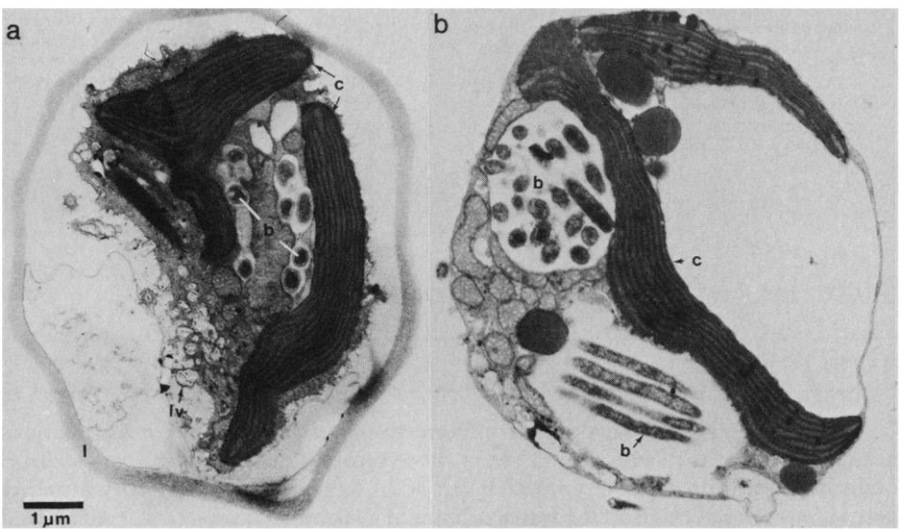
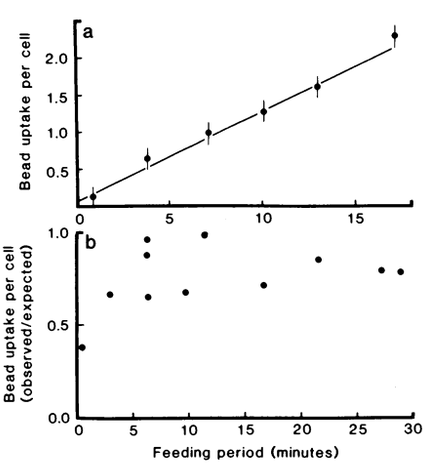


Fig. 1. Electron micrographs showing bacterial cells inside chrysophycean algae from Lac Cromwell. (a) *Dinobryon cylindricum* cell with two food vacuoles containing bacteria (b). Chloroplast (c) is also indicated. Fiber-containing vesicles (lv) are for secretion of lorica (l) (*9*). (b) Thin section of *Uroglena americana* showing two large food vacuoles.

Fig. 2. (a) Time course of bead uptake by *Dinobryon* species in the metalimnetic algal biomass peak, Lac Cromwell (3 m, 12°C). Each point represents the mean bead count, with 95 percent confidence limits, for 300 to 600 cells. Uptake rate was determined from the slope of the relation between bead content per cell (y) and incubation time in minutes (x): $y = 0.064 + 0.125x$, $r^2 = 0.99$. (b) Comparison of bead and bacteria uptake by *Dinobryon*. Experiments were performed on 28 November 1984, when a *Dinobryon* population nearly free of extraneous bacterivores was found beneath the lake ice. Each point represents a separate feeding experiment (*5*). Bacteria and beads were offered in the ratio 17.3 ± 1.2 to 1 ($n = 2$); observed uptake was 22.85 ± 2.5 to 1 ($n = 11$), so that bacteria were ingested 1.32 ± 0.11 times as readily as beads. Results are expressed as the ratio of the number of beads found inside *Dinobryon* cells to the number expected to be there were there no discrimination.



bryon ingested 0.125 bead per minute per cell (95 percent confidence limit, ±0.016), meaning that an average of three bacteria were consumed by each algal cell every 5 minutes (Fig. 2). This is equivalent to an individual *Dinobryon* removing bacteria from a volume equal to 1,500,000 times its cell volume and ingesting almost 30 percent of its weight in bacteria per day. *Uroglena* ingested 0.022 bead per minute per cell (95 percent confidence limit ±0.007). This rate is much lower than *Dinobryon*'s, both per cell and per unit of biomass.

Although microscopists have noted bacterial ingestion by *Dinobryon* previously (*6, 7*), our results show that phytoplanktonic phagotrophy is quantitatively important in nature. The grazing rates are of the same magnitude as those measured for marine microflagellates that lack photosynthetic pigments and are therefore totally dependent on external sources of carbon (*8*). Under the dim conditions of the metalimnion *Dinobryon* obtained at least 50 percent of its total carbon by bacterivory and thus at most 50 percent by photosynthesis (*9*). In other experiments in Quinn Bay, Lake Memphremagog (Quebec-Vermont), we found that *Dinobryon* removed more bacteria from the water column than the crustacean, rotifer, and ciliate communities combined (Table 1) (*10*). Such bacterivory may be a major factor in bacterial loss and a major source of carbon for some algae growing under low-light conditions in nature.

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5. Iodine-fixed samples were filtered through 105-μm Nitex screen and trapped organisms were rinsed with jets of filtered lake water. This removed most unincorporated beads, bacteria, and extraneous bacterivores such as microflagellates and ciliates. The trapped plankton were then washed onto 10-μm Nuclepore filters and mounted in 65 percent glycerin for counting. Unincorporated beads always formed less than 5 percent of the total. After bead enumeration, organisms were washed into scintillation vials and digested with Protosol (New England Nuclear) at 55°C for 12 hours before radioactivity was counted.
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cotized with 0.1 percent nicotine, after feeding to prevent regurgitation or defecation of ingested particles, preserved with Formalin, and cleared with sodium hypochlorite. All other organisms were preserved with mercuric chloride. Crustaceans were enumerated in samples obtained by towing 75-μm net four times in the epilimnion (0 to 5 m). At least 400 animals were counted per tow. At least 50 rotifers and 50 protozoans were enumerated in each of three integrated whole-water samples drawn with a Nalgene tube (inner diameter, 2.5 cm). Bead uptake was determined for 80 crustaceans, 80 rotifers, 50 ciliates, and 196 *Dinobryon* cells. Abundant genera were *Mesocyclops*, *Daphnia*, and *Bosmina* (Crustacea), *Conochilus* and *Keratella* (Rotifera), and *Halteria* (Ciliata). The *Dinobryon* population was largely *D. bavaricum* with some *D. sociale*.

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Diet-Induced Head Allometry Among Foliage-Chewing Insects and Its Importance for Graminivores

E. A. BERNAYS

Individuals of the grass-feeding caterpillar of *Pseudaletia unipuncta*, reared from hatching on hard grass, had head masses twice as great as those of caterpillars fed soft artificial diet, even though the larvae reached the same body mass. Larvae reared on soft wheat seedlings had intermediate head masses. Thus muscular effort increases muscular development in an insect, which in turn has a dramatic morphogenetic effect on head size. Size differences in the head capsules, with the correlated differences in mandibular power, have a direct effect on the ability of the insects to ingest hard foods rapidly: larger heads are adaptive for dealing with hard grasses.

JAW AND TOOTH ADAPTATIONS ASSOCIated with grass feeding occur in a number of animal groups. Most studies of grass-feeding specialists among the insects have focused on grasshoppers. Mandibles of grass-specializing grasshoppers show chisel-like incisors and flattened, grooved molar cusps, analogous to the adaptations of teeth in grazing mammals (*1*). Among caterpillars, fewer studies have been undertaken, although taxonomic studies in the genus *Spodoptera* (*2*) show that the grass specialists have mandibles with chisel-like edges while other species have pointed incisor cusps. In the study reported here, the allometric consequences of food choice were investigated.

A total of 82 grasshoppers and 76 caterpillars collected in North America and Australia were examined (*3*). Gut contents were removed and the body, head, and mandibles of each were dried and weighed. In both groups, grass specialists consistently had a greater head mass than other foliage feeders of similar body sizes (Fig. 1). Among grasshoppers the proportionality coefficients were 0.22 ± 0.04 (mean ± standard error) and 0.12 ± 0.04 for grass specialists and non–grass specialists, respectively, while among caterpillars the figures were 0.15 ± 0.04 and 0.04 ± 0.03, indicating a much greater difference in the latter group.

Relative head mass also varies intraspecifically in relation to diet. The effect of diet on head development in the grass-feeding caterpillars of the noctuid *Pseudaletia unipuncta* was studied, with some additional experiments on two acridids, *Locusta migratoria* and *Chorthippus curtipennis*. The diets were (i) artificial (extremely soft), (ii) *Poa* and *Triticum* seedlings (relatively soft C_3 grasses), and (iii) mature *Zea*, *Bambusa*, and *Cynodon* (hard C_4 grasses) (*4*).

Table 1. Effect of diet on relative head size in larvae of *P. unipuncta*. Values are means ± standard errors.

Rearing diet	Number	Head dry mass (percentage of total body dry mass)
	Experiment 1	
Triticum	20	11.9 ± 0.5
Zea	20	14.7 ± 1.4
	Experiment 2	
Artificial diet	20	8.1 ± 0.3
Triticum	23	12.3 ± 0.4
Cynodon	22	15.6 ± 1.6

Caterpillars of *P. unipuncta* were raised from hatching on one of the three diets. Relative head masses were measured in the final larval instar (*5*). Differences in head mass were extreme (Fig. 2); each group was significantly different from the others ($P < 0.005$, F test on coincidence of lines). Caterpillars reared on *Triticum* seedlings or *Zea* showed a similar pattern (Table 1), while heads from the three-diet experiment showed significant differences in morphometrics and in the surface area of attachment of mandibular adductor muscles (Table 2) (*6*). Diet had induced major changes in the caterpillars during growth.

Locusta migratoria larvae were reared on *Poa* or *Bambusa* (*7*), while *C. curtipennis* larvae were reared on *Triticum* or *Zea* (*8*). In both cases insects reared on the harder grass had relatively larger heads, although they also had lower masses than those reared on softer grasses and the exponents were significantly greater in the regression analysis. Comparison in terms of head mass as a percentage of total body mass gave values of 22 ± 1 and 17 ± 2 percent (means ± standard errors) for *C. curtipennis* reared on *Zea* and *Triticum*, respectively, and 25 ± 2 and 21 ± 2 percent for *L. migratoria* reared on *Bambusa* and *Poa*, respectively.

That grasses are difficult to chew is generally accepted, the most important feature being the parallel arrays of sclerenchyma fibers (*9*). In addition, high silica levels are presumed to cause wear of cutting and

Table 2. Head morphometrics of fifth-instar larvae of *P. unipuncta* after rearing on three different diets. Insects were of similar total body mass. Values (means ± standard errors) in each column are significantly different from one another ($P < 0.005$, t-test).

Mandible base to top of cranium (mm)	Maximum head width (mm)	Surface area of mandibular adductor muscle attachments to cranium (mm^2)
	Artificial diet	
2.01 ± 0.01 (n = 10)	1.98 ± 0.02 (n = 10)	2.8 ± 0.3 (n = 5)
	Triticum	
2.16 ± 0.02 (n = 10)	2.15 ± 0.01 (n = 10)	3.6 ± 0.3 (n = 5)
	Cynodon	
2.45 ± 0.02 (n = 10)	2.43 ± 0.02 (n = 10)	4.6 ± 0.6 (n = 5)

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