Sweet molecule	Threshold concentration T (mM/l)	'Accession efficiency' A	Moles acceding to receptor AT (mM/l)	Affinity K_m (mM/l)
Sucrose	9.6	16.71	160.4	37.4
Maltose	21.0	7.61	159.8	249.2
Lactose	28.6	5.41	154.7	716.3
Glucose	26.1	5.73	149.6	386.3
Xylose	29.0	5.19	150.5	977.8
Galactose	28.9	10.82	312.7	940.3
Fructose	15.5	20.37	315.7	124.9
Sorbitol	26.3	7.05	185.4	580.7
Xylitol	21.0	8.88	186.5	209.7

30 panellists tasted 5-ml samples (in distilled water) of each sugar (reagent grade, Sigma Chemical Co., Poole, Dorset), by the sip and swallow method, rinsing with distilled water and pausing 1 min between samples. Intensities were recorded on the Sensory Measuring Unit for Recording Flux (SMURF, i.e. a potentiometer 'dial box' connected to a Telsec Type X moving chart recorder, with which panellists turned the dial from 0 to 10 units (recorded on the chart as 0–100 units) according to the subjective intensity of sweetness). Threshold concentrations (T) were determined in a separate experiment using the method of Gregson[6]. Time/intensity plots were then determined at 5, 10, 15 and 20 times the threshold concentration of each sweetener.

A ('accession efficiency') is defined as the antilog of the intercept on the y axis obtained after plotting log intensity (i.e. log magnitude estimation) against log concentration (% w/v). Values of log A were calculated by linear regression to ensure the best fit straight line was used.

Magnitude estimation rates (MER) (units/sec) were calculated as maximum intensity/time to maximum intensity for the 4 concentrations. Affinities (K_m) were then obtained from Lineweaver-Burk type plots of reciprocal MER against reciprocal concentration (mM/l) ($-\frac{1}{K_m}$ = intercept on x axis).

'Accession efficiences' of sapid molecules may be related to their affinity for the receptor and recordings of intensity/time profiles in taste allow magnitude estimation rates (MER) to be obtained from onsets of response. Reciprocal plots of MER and concentration give Lineweaver-Burk type plots from which affinities (K_m) are calculable. The table lits K_m-values for the given sweeteners which differ from 'accession efficiences' as shown, but accord with threshold concentrations in that the sweet molecules studied are in the same order for both affinity and threshold concentration.

Previous taste studies[8,9] have illustrated molar proportionality of response and parallel gustatory effects in conformational analogues but this, to our knowledge, is the first report of molar accessibility in sweet taste chemoreception. The results are relevant to attempts to educe models of gustatory chemoreception based on occupancy theory or rate theory of stimulus/receptor interaction.

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A study on the parameters of digestion in *Periplaneta americana* L.

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Summary. An attempt was made to provide evidence for the regulatory mechanism for digestion in *Periplaneta americana* by studying possible correlations between digestive enzymes, taking midgut protease activity into consideration.

Synthesis and secretion of digestive enzymes in insects appear to be regulated by 3 possible mechanisms; a) neural, b) secretagogue, and c) hormonal. A direct stimulation by the nervous pathway seems not to be involved in most insects. In cockroaches, including *P. americana*, the innervation of the midgut is meagre[2,3] and the nerves appear to be motor ones, supplying only the gut musculature[2].

The present study was undertaken keeping the view in mind that a study of the parameters of digestive enzymes may provide clues to demonstrate the control mechanism of digestive processes. Most of the digestive processes are attributed to the midgut, and proteins are the most important constituents of the diet, therefore the determination of the midgut protease activity was chosen for the present study.

Material and methods. Adult *Periplaneta americana* of both sexes were taken from an age-regulated stock-colony maintained in the laboratory. Protease activity was determined by the method of Charney and Tomarelli[4] and protein

Table 1. Showing the effect of feeding on the midgut protease activity, after starvation for 3 days

	Normally fed	Starved (3 days)	Starved (3 days), then fed and sacrificed after 1 h	2 h	4 h	8 h	12 h
	49.0	10.0	14.5	17.5	20.0	16.5	39.5
	47.5	17.5	16.5	38.0	42.0	29.0	35.0
	35.0	14.5	28.0	27.0	17.5	25.0	27.0
	30.5	23.0	30.5	26.0	23.0	35.0	43.0
	48.0	15.75	34.0	20.0	35.5	40.5	46.5
Mean ± SD	42.00 ± 8.61	16.15 ± 4.73	24.70 ± 8.69	25.70 ± 7.95	27.60 ± 10.61	29.20 ± 9.22	38.20 ± 7.57

Table 2. Showing the effect of meal size on the midgut protease activity

Meal size range (mg)	Protease activity												Mean ± SD
0– 50	2.50	5.50	11.00	13.50	10.25	14.00	11.75	15.00	7.25	9.50	14.25	8.00	10.21 ± 3.85
51–100	19.25	29.00	27.50	10.50	25.00	23.00	30.50	21.25	22.00	31.50	15.25	26.75	23.46 ± 6.29
101–150	29.50	27.75	31.50	33.00	32.75	28.75	32.00	26.00	31.00	35.50	23.25	34.00	30.42 ± 3.52
151–200	38.75	34.00	43.25	32.00	39.50	41.00	42.00	33.50	45.25	36.00	27.00	29.50	36.81 ± 5.72
More than 200	52.00	48.00	49.00	51.00	47.00	34.00	32.50	45.50	43.00	44.50	28.75	37.00	42.69 ± 7.74

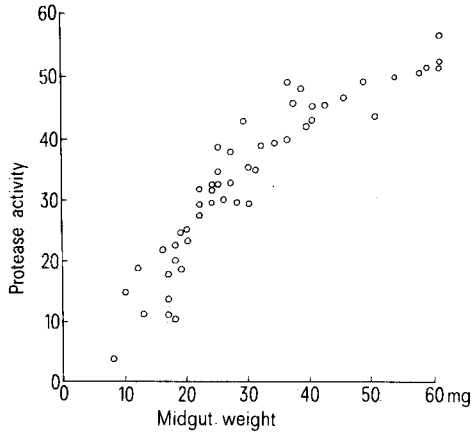

Figure 1. Showing midgut protease activity in relation to the midgut weight.

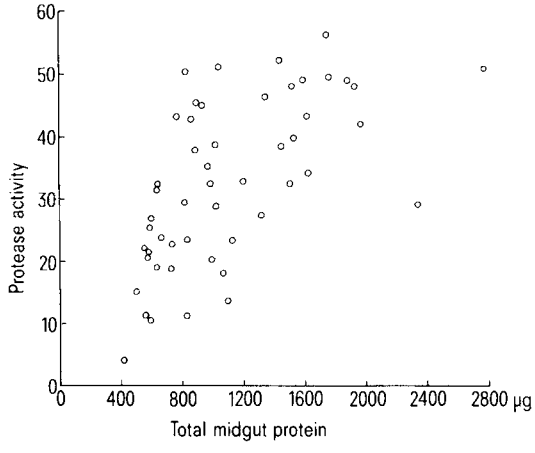

Figure 2. Showing midgut protease activity vs total midgut protein.

concentration was determined according to Lowry et al.[5]. Similar results were obtained for both male and female insects, therefore they are not given separately.

Results. In the 1st set of experiments, midgut protease activity was determined in 3-day-starved roaches, and in roaches starved then 2-h fed, sacrificed at different time intervals after feeding. It was observed that starvation caused a significant decline in the midgut protease activity which gradually increased after feeding, finally approaching normal values (table 1).

To see the effect of meal size, 3-day-starved roaches were allowed to feed on variable amounts of diet for 2 h. By weighing the insect just before and after feeding, the exact meal size was determined by the weight difference. The roaches were sacrificed on the next day for midgut protease estimation. Increase in the meal size was found to have a pronounced effect in elevating midgut enzyme activity (table 2).

Table 3. Showing the effect of feeding on diets of variable protein concentration on the midgut proteolytic activity

Protein concentration in diet	Midgut protease activity					Mean ± SD
Little	22.0	26.5	14.5	34.0	39.5	27.30 ± 9.83
Low	23.5	17.0	40.0	31.5	36.0	29.60 ± 9.34
Medium	17.0	23.5	29.0	40.0	37.5	29.40 ± 9.57
High	28.0	41.5	36.0	22.5	30.0	31.60 ± 7.34

In the 3rd set of experiments, a number of roaches of the same age and sex, in batches of 10, were starved for 3 days, fed for 2 h and sacrificed on the next day. The midgut was dissected out, blotted on filter paper, weighed and used for protease activity assay and protein determination. When the midgut protease activity was plotted against the midgut weight, it was found that there was a more or less definite linear correlation (fig. 1), whereas midgut protease activity did not show corresponding changes with total midgut protein (fig. 2).

In the last set of experiments, a series of diets differing in their protein concentration were prepared. On the basis of protein concentration estimates in relation to the protein content of the normal roach diet, such diets were categoried as having little, low, medium and high protein contents. 3-day-starved insects were allowed to feed on these diets for 2 h and they were sacrificed on the next day for midgut protease estimation. The results (table 3) demonstrate that when the substrate (protein) concentration in the diet was increased, no relative increase in the midgut protease activity followed.

Discussion. The quality and the quantity of food are supposed to be the important factors in the digestive physiology of insects. It is a general feature of animals that the digestive enzyme activity rises after feeding, reaches a maximum and then declines as the meal is digested. This has been demonstrated in many insects including *Blatella germanica*[2] and *Periplaneta orientalis*[6]. A correlation between the meal size and the midgut enzyme levels has been established in a number of insects. But roaches have not been studied in this respect, apart from *Leucophaea maderae*[7]. The present results demonstrate that in *P. americana,* feeding gradually increases midgut protease activity and the enzyme activity has a definite correlation with the meal size and the midgut weight, and thus with the amount of foodstuff in the midgut.

In many insects a secretagogue stimulation of digestive enzymes has been suggested on the basis of evidence of differential secretion elicited only by the appropriate substrate in the food. It has been demonstrated in many insects including the cockroach, *Leucophaea maderae*[8], that protease levels are related to the presence of protein in the diet and digestive tract. Gooding[9–11] in *Glossina austeni,* Garcia

and Garcia[12] in *Rhodnius prolixus* larvae and Baker[13] in *Attagenus megatoma* larvae reported a correlation between the increase in proteolytic activity and total midgut protein. Stimulation of protease and amylase activity in *Drosophila melanogaster* larvae[14] and protease and sucrase activity in *A. megatoma* larvae[15] were found to be substrate specific.

In contrast, there are number of reports which have shown the failure of substrate specific stimulation of digestive enzymes. Day and Powning[2] demonstrated that in *B. germanica,* the digestive enzymes increase irrespective of the diet, and a starchy meal did not result in a relative increase in amylase activity. It has already been reported that *Periplaneta* was unable to change its digestive enzymes from one diet to another[6]. Similarly in *Dytiscus,* fluctuations of amylase and protease occurred on water ingestion. Even in some insects in which a secretagogue mechanism has been shown to be operative, there are some apsects which can not be explained on the basis of substrate induction of enzymes and it has been suggested that protein affects digestive enzymes in general and not only the enzyme specifically acting on the protein substrate.

On the basis of the present results showing that the midgut protease activity changes are not directly related to the dietary and midgut protein (substrate) contents, a secretagogue mode of stimulation of digestive enzymes appears not to be involved, at least with regard to proteolysis. Evidence for the unlikeliness of neural involvement is already available[2,3] and therefore, a hormonal pathway is the only one which seems to be probable in the system. The direct demonstration of this 3rd regulatory mechanism needs further detailed experimentation, which is in progress.

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Simultaneous syntheses of cytoplasmic and chloroplastic ribosomal RNA during the cell cycle of *Dunaliella*

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Summary. Chloroplastic and cytoplasmic ribosomal RNA syntheses were analyzed in synchronous cultures. All 4 rRNA species are simultaneously accumulated during the light period of the cell cycle. Cytoplasmic rRNA synthesis is repressed only during the later part of the S phase.

Our previous studies on the cell cycle of *Dunaliella,* a naturally wall-less volvocale, synchronized by light-dark periods, have shown that nuclear and chloroplastic DNA syntheses occur almost completely during the light period[1]. The replication of both DNAs is simultaneous, which is not the case in some other green algae, for example *Chlamydomonas*[2] and *Chlorella*[3]. In these algae, chloroplastic DNA is synthesized a long time before nuclear DNA. Our results suggested some relationship between the DNA synthesis in the *Dunaliella* nucleus and that in the plastid. In the present work, our intent was to determine the chronology of the ribosomal RNA transcription during the cell cycle in the nucleus and the chloroplast to point out other possible temporal relationships between metabolic processes in the two organelles.

Materials and methods. Dunaliella bioculata (Volvocales), a unicellular green alga, was grown on a mineral growth medium[4] at 24 °C, under a 8/16 light-dark cycle at 10 klx. Under these conditions, synchronous divisions occurred every 24 h during the first part of the dark period[4]. For radioactive labelling, the cells were incubated for 1 h with $Na_2\ ^{32}PO_4$ (0.3 μCi/ml in the culture medium) at different times during the cell cycle. The cells were then harvested by centrifugation at 5000 × g for 10 min at 4 °C. The pellet was washed twice in the culture medium and frozen for later use.

Ribosomal RNA extraction followed Laulhère and Rozier's technique[5]. The rRNA was analyzed by electrophoresis on 2.5% polyacrylamide gel according to Loening[6]. The radioactivity was detected by liquid scintillation counting in Aqualuma or in Lipoluma after eluting the gel slices in Lumasolve.

Results. The ribosomal ribonucleic acid from *Dunaliella bioculata,* separated by electrophoresis, showed 4 species (fig. 1) although Ralmsdorf et al.[7] showed only 3 species in *Dunaliella* sp.: 26 S, 23 S and 17.5 S. As compared with the results obtained from very similar algae such as *Chlamydomonas*[8], we called these 4 species 25 S and 18 S (cytoplasmic rRNA), 23 S and 16 S (chloroplastic rRNA). These designations are intended only for identification of the RNA components and do not imply accurately measured sedimentation coefficients or electrophoretic mobilities. It was difficult to obtain undegraded 23 S RNA (as with numerous green algae) and the absorbancy ratio between the peaks of 23 S and 16 S RNA was not the same for each extraction (1–1.8) even though the ratio between the peaks of 25 S and 18 S RNA was always close to the expected value (about 1.9). Galling[9] reported the same degradation in *Scenedesmus* and *Chlorella,* as did Dazy[10] with *Acetabularia.*

In a previous study[4], we pointed out that the total cellular rRNA is accumulated during the light period of the cell