slightly (cf. 3–4 and 7–8), whereas ganglionic 'off' activity disappeared completely. Thus, a small decrease in light, regardless of the initial absolute intensity, is apparently not a sufficient stimulus for the ganglionic 'off' response although behavioral shadow reflexes occur in response to step decreases of as low as 0.5% [10].

'On' response activity appears to be predominant in the lateral lobe of the ganglion, a functional optic lobe [12], and responds without decrement at frequencies of 5–6 Hz (figure 2B). Mechanically-evoked activity from the mantle also appears not to enter the lateral lobe (figure 1C). If shadow response behavior relies on activity in 'off' fibre tracts [3], our results suggest that these fibres synapse at sites within the ganglion other than in the lateral lobes, perhaps directly in the dorso-central (motor) lobes. The function of the lateral lobe, suggested in part by its intricate glomerular structure [12], is apparently concerned with more complex behaviors such as orientation to boundaries in the visual field [10].

An alternative interpretation of the present results would suggest that 'on' activity, incorporating integration of both spatial and temporal features of the stimulus, may trigger the shadow reflex. Some evidence for this hypothesis exists since, in our experience and from other experiments [10], shadows produced by uniform darkening of the visual field are typically ineffectual. Rather, an effective stimulus for valve closure always involves movement, a situation in which the more predominant, rapidly responding 'on' receptors would be re-illuminated following transient shadow periods. Further experimentation is needed to resolve the central mechanism of the shadow response.

The effect of a low protein diet on the adipocytes of rat perigenital fat

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Summary. Weanling rats were fed a low protein diet for 6 weeks and their weights were 50% less than controls. There were significantly fewer adipocytes per μg adipose tissue, but estimates of the number of adipocytes per rat indicated that the diet had much less effect on adipocyte number than on b.wt.

The physiological control of adipocyte production is not well understood, but evidence points to the involvement of the hypothalamus [1], possibly related to growth hormone [2]. By sampling at various ages it has been demonstrated that adipocyte production continues for some time after weaning [1,3–5], and during this time rats may be susceptible to the influence of nutrition on the endogenous control of adipocyte production. For example, a high fat diet causes increased adipocyte number [6], and starvation has been shown to inhibit [5] or to have no effect [4] on adipocyte production, and one report showed no change in adipocyte number after underfeeding [7]. Adipocyte number is also affected by preweaning nutrition and adult rats which were suckled in small litters had more adipocytes than those suckled in large litters [3], and this phenomenon was also demonstrated in genetically obese rats [8]. When rats are fed a low protein diet, their growth is stunted, but they have the capacity to grow to nearly normal weight when given a good diet later in life [9,10]. The purpose of the present work was to investigate the response of rat adipose tissue to a low protein diet, with particular reference to the number of adipocytes in the tissue. Rats were also suckled in different size litters to confirm whether the adipocyte counting method was sensitive enough to detect a well established difference in adipocyte number.

Materials and methods. Sprague-Dawley rats were suckled in litters of either 4 or 16, during which time the dams were fed a nutritionally adequate diet [11]. For 6 weeks after weaning, the small-litter rats were fed a high protein diet and half of the large-litter rats were fed the high protein diet (controls) and the rest were fed a low protein diet. Both diets contained (g/kg) 40 corn oil, 40 mineral mix and 20 of vitamin mix, with either 100 or 250 casein and

Weights, fat contents, adipocyte concentration and estimates of number of adipocytes in 9-week-old rats

		Small litter* HP Diet	Large litter** HP Diet	LP Diet
Number of rats	(male)	4	5	5
	(female)	4	6	6
Weight (g)	(male)	313 ± 5	212 ± 4	98 ± 4
	(female)	201 ± 9	169 ± 6	95 ± 10
Fat (g)	(male)	29 ± 3	12 ± 1	11 ± 0.5
	(female)	16 ± 1	10 ± 1	12 ± 1.5
Adipocytes μg^{-1}	(male)	6.13 ± 0.88	9.19 ± 0.97	6.55 ± 0.61
	(female)	7.39 ± 0.42	8.94 ± 0.82	7.41 ± 1.24
	(both sexes)	6.76 ± 0.49	9.05 ± 0.63	7.02 ± 0.71
Adipocytes $\times 10^6$ per rat	(male)	200 ± 27	142 ± 17	102 ± 10
	(female)	147 ± 30	107 ± 9	102 ± 8

Mean values ± SE. * Litter of 4 pups; ** litter of 16 pups; fed at high protein (HP) or low protein (LP) diets after weaning.

800 or 650 starch. At 9 weeks of age, the rats were killed. Samples of fat from the left epididymal or periovarian pad were frozen in liquid nitrogen. Adipocytes of a 20 mg sample were counted by the method previously described, using the FN Coulter Counter[1]. Briefly, the sample was incubated in a solution of 20 g dm^{-3} osmic acid for 3 days at 37 °C and homogenized to release the solidified adipocytes for counting. Results were expressed as number of adipocytes per µg of adipose tissue[12]. Approximately 150 mg samples of fat pad were homogenized with 2:1 chloroform:methanol for 2 min and the fat so extracted was weighed to constant weight. 5 g samples of dried and ground carcass were analysed for fat by Soxhlet using 60–80 petroleum ether. From this data, the total number of adipocytes in the rat were estimated.

Results and discussion. The low protein diet caused a 50% stunting of growth, while the small-litter rats were 35% heavier than the controls. Because adipocyte concentrations were almost the same for males (7.4 μg^{-1}) and females (7.9 μg^{-1}), the results for both sexes were pooled for Student's t-test. Rats fed the low protein diet had a lower concentration of adipocytes than the controls ($p < 0.05$). Also the concentration of adipocytes of the small-litter rats was less than the controls ($p < 0.02$). Expressed in another way, both the small-litter and low protein fed rats had larger adipocytes than the controls. Calculation of the numbers of adipocytes in the whole rat always requires an assumption that the adipose tissue analyzed is representative of all the many different sites. It is known that this assumption is not strictly true, so the estimates of total adipocytes must be treated with caution and were not statistically analyzed. However, it is noteworthy that the low protein diet caused a marked stunting in growth, but there was much less effect on the number of adipocytes in these rats. It appears that the endogenous control of adipocyte production was not much affected by this particular diet, and the rats were able to store approximately as much fat as the controls. The data also suggests a sex difference in response to this diet, and this requires further investigation. The small-litter rats had a greater number of adipocytes than the controls, which is consistent with the findings of Knittle and Hirsch[3]; also the sizes of adipocytes quoted by them for the same strain at 9 weeks old were similar for small (4) and large (22) litter male rats compared to the figures obtained in this experiment.

In conclusion, it appears that most of the control number of adipocytes are present in rats fed a low protein diet before the period of 'catch up' growth, and this could help to explain how the weight can increase so rapidly when a good diet is given to previously malnourished rats.

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Seasonal monoamine changes in the central nervous system of *Mytilus edulis* (Bivalvia)

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Summary. The levels of serotonin, dopamine and norepinephrine exhibit seasonal changes in the central nervous system of Mytilus edulis. These monoamines were higher during the summer and lower during the winter.

Variations in serotonin (5-HT) levels have been demonstrated in rat[1], cats[2], turtles[3] and gastropod molluscs[4,5]. Seasonal variations in 5-HT levels have been demonstrated in mammals[6,7] and gastropod molluscs[4,5]. Various investigators have suggested that these seasanol monoamine changes may also occur in bivalve molluscs[5,8–10]. However, a study directed at this phenomena has not been performed until now.

Materials and methods. Subtidal M. edulis were collected from the shores of Long Island Sound at New Rochelle, N.Y. The animals were randomly chosen on or about the 18th day of each month for a year. Within 40 min from the time of collection the total central nervous system (CNS) (2 cerebral, 2 pedal and 2 visceral ganglia) of 4 animals was excised after being frozen with dry ice chips. The CNS was then subjected to the extraction procedure or was stored for not more than 3 days, frozen over P_2O_5. The extraction procedure was carried out in the cold according to the method of Shellenberger and Gorgon[11]. Dopamine (DA) and 5-HT were quantified spectrofluorometrically according to their method, while norepinephrine (NE) was quantified by the method of Anton and Sayre[12].

Results and discussion. The CNS of M. edulis contains 5-HT, DA and small amounts of NE. The levels of 5-HT in M. edulis have been reported to vary considerably[13]. This varience can now be attributed at least in part

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