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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to seasonal differences in the endogenous 5-HT levels. Animals sacrificed at different time period during the same day did not display the large variation demonstrated in the yearly study, but did display a variation of from 5 to 10% of the mean daily value.

In examining the 5-HT levels for the year it now seems quite clear that the levels changed dramatically during the course of the experiment (table). Summer levels of 5-HT were approximately twice those found during the winter. During August and December the catecholamine content was also determined for the same animals in addition to estimating 5-HT levels. During August the DA content was 37.25 ± 0.6 μg/g (mean ± SEM) and NE was 3.57 ± 0.52 μg/g as compared to December when the values dropped to 15.55 ± 0.52 and 2.10 ± 0.11, respectively. These values were found to be statistically significant ($p < 0.005$). The DA and NE levels varied in the same manner as 5-HT, that is high during the summer and low during the winter. The summer values also are approximately twice those found during the winter. In August and December the amount of 5-HT to DA was 1.5 and 1.8, respectively. Since these values remained relatively constant this may suggest an interrelationship between 5-HT and DA as demonstrated for these animals[13].

In M. edulis York and Twarog[9] noted changes in the 5-HT content of the pedal ganglia during March and April (5.4–8.6 to 26–42 μg/g). The present study also demonstrated a sharp increase in the 5-HT levels during this time period. Therefore studies involving monoamine metabolism must be aware of these level changes which may also influence the organisms sensitivity toward endogenous and exogenous agents. These seasonal changes may play a dominant role in the organisms behavior pattern[5]. The reproductive cycle of the animal consists of several seasonal phases: development of resting gonads (fall), gametogenesis (winter), spawning (spring), rapid gametogenesis (early summer) and resting state[14]. The time sequence of the above cycle which is for the English coast can vary from habitate to habitate. In Long Island Sound where our animals were collected the cycle is very similar[15]. Metabolic activity is also seasonal, glycogen accumulates during nonreproductive periods[14] and falls in the winter. Lipids behave in an opposite manner[16]. Studies of environmental factors affecting these cycles are varied and often contradictory. However, temperature changes and neurosecretory activity are known to play important roles[17]. Recently, short-term temperature changes have been shown to alter monoamine metabolism in the CNS of M. edulis[18]. Direct correlation of our findings to the circannual behavior of M. edulis can not be made at this time. However, the regulatory activity of biogenic amines in other organisms would strongly suggest interrelationships. The authors are preparing a more detailed report on factors which may modify these seasonal monoamine changes (temperature, photoperiod and food availability).

Serotonin (μg/g ± SEM) was determined spectrofluorometrically for the CNS (2 cerebral, 2 pedal and 2 visceral ganglia) of M. edulis

Month	N	Serotonin (μg/g ± SEM)
January	8	25.10 ± 2.71
February	4	26.96 ± 2.11
March	4	32.17 ± 3.85
April	4	41.98 ± 1.22*
May	4	48.15 ± 1.02**
June	4	53.13 ± 1.71**
July	4	51.74 ± 3.14**
August	4	57.28 ± 2.49**
September	4	48.90 ± 1.13*
October	4	44.80 ± 1.51*
November	4	35.71 ± 2.70***
December	4	28.97 ± 2.64

N is the number of animals assayed. Significance was determined by a one-tailed Student's t-test. Comparisons for significance were made by comparing January to the rest of the months. * $p < 0.005$, ** $p < 0.001$, *** $p < 0.05$.

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Non-synaptic chemical neurotransmission[1]

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Summary. Images with apparently gemmulofugal polarity in the EPL of the olfactory bulb are the result of sectioning, along misleading planes, gemmulopetal synapses containing postsynaptic vesicles. Unless one accepts a bidirectional conduction for chemical synapses, the internal granule cells lack actual gemmulofugal synapses and the neurotransmitter contained in their vesicles must act at non-synaptic membranes.

It is generally accepted that most synapses permit transneuronal signalling by means of localized chemical reactions leading to specific subsynaptic changes in ionic permeability. But, does this necessarily entail that that chemical neurotransmission should be associated exclusively with the presence of synapses? It has been reported that there is a striking discrepancy between the the large number of synaptic vesicles present in the gemmules of the internal granule cells of the olfactory bulb and the scarcity or, very likely, total absence of gemmulofugal synapses[2]. In this region, gemmulopetal synapses can be easily identified (figure 1, black arrows) because of the selective vesicular accumulation on the side of the mitral or tufted profile. However, many synapses can be seen in the external plexiform layer with clusters of vesicles apposed to both sides of the junction