ses in quantal size we observed. Noise analysis of the end-plate response to ACh[6] showed that stimulation did not produce detectable changes in the single channel lifetimes or conductances (W. Van der Kloot, in preparation).

We measured the size of quanta released by nerve stimulation in high Mg^{2+}, low Ca^{2+} Ringer, in which the probability of a quantum being released by the stimulus is less than one. In preparations pretreated for 2 h in hypertonic NaCl Ringer, the size of the quanta released spontaneously and following stimulation were of similar sizes. In preparations pretreated with hypertonic gluconate Ringer, some of the largest quanta may only be released spontaneously.

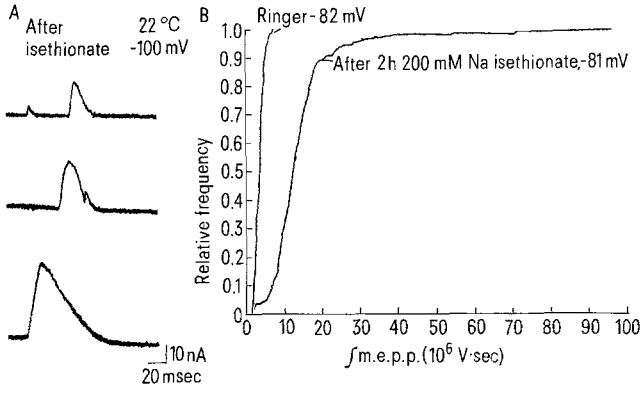

Figure 2. *A* A sartorius muscle was soaked for 2 h in a Ringer containing 200 mM Na isethionate (replacing the 120 mM NaCl). Then it was returned to normal Ringer and voltage clamped at −100 mV. The upper trace shows one of the smaller currents. The lower trace shows one of the largest. Note the slow rise of the large current. The amplifiers were AC coupled, which is responsible for the undershoot of the base line following the huge m.e.p.c. *B* In another experiment a microelectrode was inserted at an end-plate and the areas of 100 m.e.p.p.'s were measured (left). Then the preparation was transferred to Ringer containing 200 mM Na isethionate for 2 h. After the return to Ringer the areas of 100 m.e.p.p.'s were measured. The electrode remained in the fiber throughout the experiment; the resting potential decreased by 1 mV.

All of the treatments that elicit an increase in quantal size involve a period in which many quanta are released from the nerve terminal. We speculate that following quantal release at a high rate subsequent quanta contain an increased amount of ACh. An obvious hypothesis is that stimulation increases the size or the ACh content of synaptic vesicles. Anions may affect the quantal size by altering the uptake of ACh into vesicles. Obviously a mechanism that changes quantal size following activity could play a role in the physiology of the neuromuscular junction. To demonstrate the effect unequivocally we stimulated for long periods, and the changes were several-fold. We have found that stimulation for 1 h still produces large increases in quantal size, but have not yet studied shorter periods of stimulation. Much smaller changes in quantal size could be physiologically significant at other synapes. If a similar mechanism is found at central synapses it might play a role in the modification of behavior by experience.

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Influence of diet on plasma tryptophan and brain serotonin levels in mice

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Summary. Groups of mice were maintained for up to 78 weeks on tryptophan restricted, protein restricted and control diets. Plasma tryptophan levels were significantly reduced by both forms of dietary restriction. Brain serotonin levels were significantly reduced only in mice on the tryptophan restricted diet, but not for mice on the protein restricted diet. The protein-restricted diet contains less of the large neutral amino acids which compete with tryptophan to enter the brain. It is known that protein restriction and tryptophan restriction extend lifespan. The results presented here suggest that extension of lifespan and lowering of brain serotonin are not related.
Key words. Mice, brain; mice, plasma; brain, mouse; plasma, mouse; serotonin, brain; tryptophan, plasma; brain serotonin; dietary restriction.

Dietary restriction is the most effective means of extending the lifespan of rodents[1-3]. Several methods of restriction have produced significant life-extension. These include reduction of total food intake, reduction of protein intake, and reduction of tryptophan intake. For example, mice on a low (4%) protein diet live 39% longer than control mice on a 26% protein diet[4]. Similarly rats on a low tryptophan diet live 20% longer than controls[5,6]. Based on these observations, the question arises as to whether low protein and low tryptophan diets produce life span extention by similar biological mechanisms.

Comfort[7] has postulated that both dietary regimes act by lowering the level of tryptophan available for brain serotonin synthesis. It is hypothesized that there is a 'biological clock' in the brain controlled by serotonin which regulates growth, development, aging and death[7-9]. If so, any intervention that altered brain serotonin level could act to reset the 'biological clock' and alter lifespan.

In view of these considerations, the purpose of the present study was to compare the level of brain serotonin and of its precursor, plasma tryptophan in mice on protein restricted vs. tryptophan restricted diets. If both diets do act by the same mechanisms, one might expect to find similar levels of brain serotonin in mice on both diets.

Materials and methods. 3-week-old male Swiss albino mice, obtained from Canadian Breeding Farms, St. Constant, Quebec, were fed Purina rat chow until they attained a b.wt of approximately 20 g (4 weeks of age). The control group was then placed on a diet containing 26% protein (TD 78071), one experimental group was placed on a protein restricted diet containing 4% protein (TD 78072) while the second experimental group was placed on a tryptophan restricted diet (TD 78464). All diets were obtained from Teklad Company, Madison, Wisconsin, and their composition is described in earlier papers[4-6]. The control diet contained 0.47% tryptophan, the low protein diet contained 0.07% tryptophan and the tryptophan restricted diet contained 0.08% tryptophan. Since the minimum requirement for tryptophan in mice is 0.10% both experimental diets were tryptophan deficient[10]. Plasma tryptophan measurements were carried out using the procedure of Denkla and Dewey[11]. Brain serotonin measurements were carried out using the method of Barchas et al.[12]. These measurements were carried out on five mice on each diet at 8, 12, 24, 36, 52 and 78 weeks of age. The mice were sacrificed by cervical dislocation between 10.30 and 12.30, since this is the period when serotonin levels are highest in rats[13].

To determine whether there were statistically significant differences between groups, a one-way analysis of variance (ANOVA) was performed followed by the least significant difference test at a 95% or 99% confidence level. To determine whether there were age related declines in any parameter measured, the product-moment correlation coefficient was determined[14].

Results. The data presented in table 1 show that placing mice on either the tryptophan restricted diet or the 4% protein diet resulted in lowered levels of plasma tryptophan. The tryptophan restricted diet produced the most marked effect by lowering the plasma tryptophan values to 34–45% of control values. The protein-restricted diet reduced plasma tryptophan levels to a lesser extent; to 44–83% of control values.

The fact that measurements of plasma tryptophan were carried out at four different ages allows us to evaluate age related changes in this parameter. The values for the control mice and for the tryptophan restricted mice show very little change with age. In contrast, there was a decline in plasma tryptophan levels with age for mice on the protein restricted diet. The product-moment correlation coefficient was −0.99, providing clear evidence of a linear age-related decline in plasma tryptophan for this group of mice.

Brain serotonin measurements for mice on the three groups are presented in table 2. The level of brain serotonin is significantly lower in mice on the tryptophan restricted diet as compared to the control group. It ranges from 52% to 82% of control values. In contrast, there is no significant difference in brain serotonin levels between the protein restricted and control animals.

Discussion. The results presented here show that the two forms of dietary restriction used in this study both caused a significant decrease in plasma tryptophan level in mice. It is well established that a tryptophan deficient diet will produce a decline in plasma tryptophan levels within 1–2 days in rats[15-18]. The results show that brain serotonin levels were significantly lower in the tryptophan restricted mice as compared to either the protein restricted mice or to the control mice. To explain why brain serotonin levels are not reduced to an equal extent by the two restricted diets, it is necessary to consider that one variable in this study is the carbohydrate content of these two diets. The protein restricted diet contains 82.1% carbohydrate while the tryptophan restricted diet contains 57% carbohydrate. Fernstrom and Wurtman[19] have demonstrated that ingestion of carbohydrates will cause an increase in brain serotonin. The fact that the protein restricted diet in the present study had a higher carbohydrate content than the tryptophan restricted diet could thus explain why brain serotonin levels remained at a higher level in the group of mice of this diet.

The transport of amino acids into the brain is a second variable which should be considered in attempting to explain the differences in brain serotonin levels observed here. It is known that tryptophan competes with five other neutral amino acids for carrier molecules to transport it across the blood-brain barrier[20,21]. In the protein restricted diet, where all amino acids are low, there would be less competition for tryptophan to enter the brain. Both these variables could ultimately be related to the effects of the two diets on insulin secretion. The higher levels of carbohydrates in the protein restricted diet could act to increase insulin secretion, which would in turn act to lower the plasma levels of the large neutral amino acids which compete with tryptophan to enter the brain[22].

The results presented here show that protein restriction and tryptophan restriction do not lower brain serotonin to an equal extent. This may be due to the intervention of other factors in the diet; carbohydrate content and competition from neutral amino acids. Both diets are known to extend lifespan. The results of the present study do not allow us to conclude that there is a common mechanism by which these diets act, mediated via brain serotonin levels. The life-extension observed with the diets may depend more on other hormonal or neurotransmitter mechanisms[23].

Table 1. Comparison of plasma tryptophan levels for control mice on a control 26% protein diet with those of mice on a tryptophan restricted diet and those on a protein restricted diet. The percentage of the control value is indicated. Plasma tryptophan (nmoles/ml). Mean ± SD (N = 5).

Age (weeks)	Control	Tryptophan restricted	% of control	Protein restricted	% of control
*** 8	78.0 ± 13.0	*34.9 ± 1.5	(45%)	**65.1 ± 8.5	(83%)
12	94.1 ± 28.5	*38.7 ± 8.3	(41%)	67.4 ± 21.4	(72%)
***36	94.3 ± 7.3	*32.2 ± 12.7	(34%)	*57.3 ± 6.9	(61%)
78	97.5 ± 11.6	*41.9 ± 16.6	(43%)	*43.3 ± 20.9	(44%)

* Significantly different from control ($p < 0.01$). ** Significantly different from control ($p < 0.05$). *** Significant difference between the two restricted groups ($p < 0.01$).

Table 2. Comparison of brain serotonin levels for control mice on a control 26% protein diet with those of mice on a tryptophan restricted diet and those on a protein restricted diet. The percentage of the control value is indicated. Brain serotonin (nmoles/g). Mean ± SD (N = 5).

Age (weeks)	Control	Tryptophan restricted	% of control	Protein restricted	% of control
8	1.88 ± 0.14	*1.32 ± 0.28	(70%)	2.23 ± 0.08	(119%)
12	2.17 ± 0.28	*1.37 ± 0.13	(63%)	2.10 ± 0.27	(97%)
24	2.82 ± 0.28	*1.46 ± 0.39	(52%)	2.49 ± 0.42	(88%)
36	2.13 ± 0.35	*1.35 ± 0.21	(63%)	2.14 ± 0.15	(101%)
52	1.92 ± 0.75	1.57 ± 0.35	(82%)	1.55 ± 0.06	(81%)
78	1.96 ± 0.23	*1.45 ± 0.29	(74%)	2.25 ± 0.18	(115%)

* Significantly different from control ($p < 0.01$).

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Electrical responses to taste chemicals across the dorsal epithelium of bullfrog tongue

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Summary. Stimulation of the bullfrog tongue with taste chemicals produced a slow change in transepithelial potential difference across the dorsal epithelium. The potential profile was in many respects similar to that of the intracellularly recorded potential changes in taste cells and to the activity of taste fibers in frogs.
Key words. Bullfrog tongue; dorsal epithelium; taste chemicals; transepithelial potential difference.

It has been shown that the dorsal epithelium of the dog tongue immersed in Krebs-Henseleit solution transports Na and Cl ions actively, producing a transepithelial potential difference across the tissue, which is increased by application of hypertonic NaCl solution to the mucosa and is associated with decreased resistance of the tissue[2].

In the present study, we show that the dorsal epithelium of the bullfrog tongue in contact with Ringer's solution does not develop a transepithelial potential difference, but responds to various chemicals on the mucosa in association with changes of the potential difference and of the tissue resistance. The potential change causes a change of passive transport of some ions through the tissue and may play some role in the process of taste reception.

Materials and methods. Bullfrog tongue *(Rana catesbeiana)* was isolated, and the dorsal epithelium was dissected from the underlying muscle. The tissue was mounted vertically on an apparatus designed to isolate the mucosal and the serosal surfaces (each 1 cm^2, elliptic in shape), using a modified Ussing chamber[3]. Both surfaces of the tissue were continuously perfused with aerated Ringer's solution (NaCl 112, KCl 2, $CaCl_2$ 1.8, and $NaHCO_3$ 2 mM per liter). To record the potential difference, agar-1 M KCl electrodes with an Ag-AgCl wire were placed immediately below each surface, and connected to an electrical recording apparatus via a high impedance amplifier. For stimulation, NaCl, acetic acid, quinine hydrochloride or sucrose mixed with Ringer's solution were applied to the mucosa for 50 sec instead of Ringer's solution.

Results and discussion. Only a negligible transepithelial potential difference (−1.3 ± 2.1 mV, serosa grounded) was observed across the tissue in contact with Ringer's solution alone, compared with the frog skin (−76.3 ± 18.9 mV) and the bladder membrane (−84.1 ± 17.3 mV), which were measured using the same apparatus. These findings suggest that active ion transport in this tissue is ineffective compared with transport in frog skin[3], toad bladder[4] and dog tongue[1,2].

After adaptation to Ringer's solution, a stimulation of the mucosa with NaCl, acetic acid or quinine produced a slow change of the potential difference; in the mucosa the change was negative to the grounded serosa in response to NaCl, acetic acid and quinine (NaCl-, acetic acid- and guinine-responses), while with sucrose the mucosa became positive (sucrose-response). Resting potential was restored upon withdrawal of stimulants within 1–10 min as shown in figure 1. These responses, characteristic of the agents and their concentration, resembled those

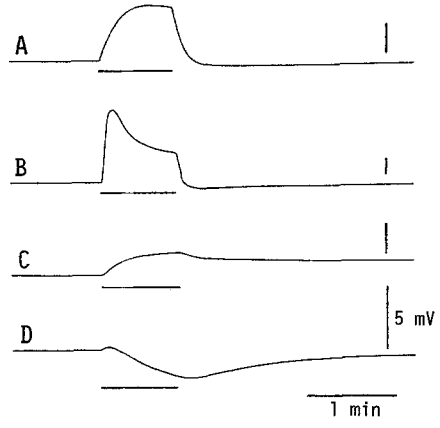

Figure 1. Records showing changes in transepithelial potential difference between the mucosa and the serosa of the same dorsal epithelium in bullfrog tongue, appearing when stimuli were applied to the mucosa during a period presented by a horizontal bar in each record. Stimulating chemicals mixed with Ringer's solution applied to the mucosa were 0.2 M NaCl (A), 5 mM acetic acid (B), 5 mM quinine (C) and 0.5 M sucrose (D). Upward direction indicates the mucosa electrically negative to the serosa, grounded, and vice versa. Vertical bars in all records show 5 mV.