difference compared with their controls, but both normal male groups show significantly higher values than the rest (e.g. M1 vs C1: $0.005 > p > 0.001$; M1 vs C2: $0.05 > p > 0.025$). The female and castrated male groups have similar values.

Discussion. The overall picture is interpretable as pointing to a male hormonal influence as playing a role in the induction of STS activity in the mouse liver. This conclusion can be reached despite the methodological limitation that enzyme activities cannot be assessed before and after hormone treatment in the same animal, in view of the tissue assayed. Certainly the development of more sensitive enzyme assays, for example, microtechniques that would allow tests to be done on small amounts of tissues such as whole blood, would be of help. In addition, it is probable that the dose of testosterone used was not the most effective, considering also the long interval between castration and the execution of the experiments reported here.

In our experiments, the effect of endogenous male hormones on enzyme induction is not apparent, when considering the absolute levels of STS in castrated and normal males: in fact the castrated group have higher – though not significantly so – mean values than normal controls. However, the situation is reversed when the enzyme activity is expressed in relation to the marker enzyme α-gal. Such a way of looking at STS activity (and that of araC) is especially appropriate as the *α-gal* gene is subject to inactivation when one of the female X chromosomes is randomly inactivated. Here, females and castrated males have similar ratios, and these are lower than in entire males. In each set, the ratios rise in those treated with testosterone injections, compared with the control animals. The effect of exogenous testosterone on induction of the enzyme is evident. It is to be noted that the effect on the castrated mice shows wide variation in response, and this could be due to an altered metabolic state after castration. These results are barely evident statistically in the present study, and this is compounded of small sample size and individual variation of enzyme levels, as well as, possibly, other factors discussed above. Finally, a relevant question concerns the role of testosterone in enzyme induction. By analogy to β-glucuronidase induction in the murine kidney, it is not implausible to suggest a form of regulatory action at the level of transcription. Confirmatory evidence for this would require studies of mRNA specific for the protein concerned, and information of this nature will be of importance in our understanding of the regulation of gene expression at this locus.

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Methylation of hippocampal phosphatidylethanolamine and proteins during long-lasting potentiation

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Summary. Whereas the monomethylation of hippocampal phosphatidylethanolamine is decreased following the induction of long-lasting potentiation of the CA_1 population spike, carboxymethylation of proteins is unaffected.

Key words. Rat hippocampus; hippocampus, rat; potentiation, long-lasting; carboxymethylation, protein; methylation, phospholipid; phosphatidylethanolamine.

Long-lasting potentiation (LLP) of synaptic transmission in the hippocampus that occurs following a brief high frequency tetanic stimulation of the input has been investigated in several laboratories as a model for synaptic plasticity[2–6]. Although there is evidence for the involvement of both presynaptic[7–10] as well as postsynaptic[11–13] elements in the process, recent findings from our laboratory[14,15] indicate that an increase in the number of glutamate receptors cannot account for LLP. Since it has been suggested that methylation of proteins and phospholipids at presynaptic membrane sites could alter the function of the membrane[16,17], experiments were done to observe whether the induction of LLP in hippocampal slices is accompanied by changes in the capacity of the membrane to be methylated.

Materials and methods. LLP-induction. Transversely sectioned rat (Sprague-Dawley, 100–150 g) hippocampal slices were prepared for electrophysiological recordings as described in a recent paper from this laboratory[18]. The slices were continuously perfused with a modified Ringer's solution (NaCl, 120 mM; KCl, 3.1 mM; NaH_2PO_4, 1.3 mM; $MgCl_2$, 2.0 mM; $CaCl_2$, 2.0 mM; $NaHCO_3$, 26.0 mM; dextrose, 10.0 mM) bubbled with carbogen (95% O_2, 5% CO_2). The temperature of the bath containing the slices was maintained at 32 ± 0.5 °C. The pH of the medium was 7.4 and the flow rate was 3 ml/min. Slices were allowed to equilibrate for at least 1 h before any recordings were made. A concentric bipolar metal stimulating electrode (SNEX 100, Rhodes Electronics) was positioned in the Schaffer collateral axonal region and a glass recording microelectrode (4 M NaCl, tip 2–3 μm; 1 MΩ) was positioned in the CA_1 cell body area of the hippocampus. Population spikes in the CA_1 area were evoked at 0.1 Hz by stimulation of the Schaffer collaterals. Stimulation strengths were adjusted to elicit about 1 mV population spike to allow room for potentiation. A tetanic stimulation (100 or 150 Hz for 1 sec) was applied and the post-tetanic population spike was followed for 10 min. If a stable LLP (at least 2-fold increase in size; 205–640%) was observed the slices were used for methylation experiments. Control slices were those that were not subjected to the tetanic stimulation. In each experiment the controls and the LLP-induced slices were taken from the hippocampus of the same animal. For neurochemical experiments 3–5 slices were pooled.

Protein carboxymethylation. This was done essentially according to the method described by Diliberto, Viveros and Axelrod[19]. The labeled methyl group of radioactive S-adenosyl methionine which is transferred to carboxylic groups of proteins at pH 6 is hydrolyzed in alkaline solution to methanol and extracted by a mixture of organic solvents. Slices were homogenized in 50 mM sodium acetate buffer. An aliquot of the tissue (100–150 μg protein) was added to an assay medium containing 50 mM sodium acetate buffer pH 6.0 (final concentration 50 mM) and about 0.1–0.2 nmole of S-adenosyl [C^3H_3]methionine (SAM, sp. act. 76 Ci/mmol; Amersham). The final volume was 0.1 ml. Incubation was done at 37 °C for 15 min and the reaction was terminated by addition of 1 ml of 10% TCA. After centrifugation the pellet was incubated for 15 min at room temperature with 0.5 ml of 0.5 M sodium borate buffer at pH 10 containing 1% methanol. The reaction medium was extracted with 3 ml of a mixture of toluene and isoamyl alcohol (3:2 v/v). 1 ml of the organic phase was counted directly by liquid scintillation spectrometry (Phillips 4700). A second aliquot of 1 ml was evaporated for 1.5 h at 80 °C in an oven and radioactivity determined. The difference in radioactivity was used as a measure of methanol formation and, therefore, of carboxymethylation of proteins. The blank consisted of incubations in the absence of the homogenate or in presence of heat denatured homogenate.

Phospholipid methylation. The methylation of phospholipids was measured at pH 8 by the incorporation of tritiated methyl groups of radioactive SAM into lipids which was extracted by chloroform-methanol mixture as described in detail by Crews et al.[16]. The slices with and without LLP were homogenized in 25 mM Tris glycylglycine buffer (pH 8) and aliquots containing 0.1–0.2 mg protein were added to an incubation medium (at 4 °C) having the following final composition: Tris-glycylglycine buffer (pH 8), 25 mM; $MgCl_2$, 2 mM and S-adenosyl [C^3H_3]-methionine (2 μCi), < 3 μM. Final volume of the incubation medium was 0.1 ml. Incubation was done at 37 °C for 30 min and stopped by the addition of 1 ml of 20% neutralized-Na-Tris (pH 7). After 10 min on ice, the samples were centrifuged at 30,000 ×g for 10 min. Phospholipids in the pellet were extracted by vigorous shaking with 2 ml of chloroform-methanol mixture (2:1 v/v) containing 50 μg/ml of the antioxidant butylated hydroxytoluene. The extract was washed twice by shaking with 2 ml of 0.1 M KCl in 50% methanol followed by centrifugation at 2000 × g for 5 min. An aliquot of the chloroform phase was transferred to a scintillation vial and evaporated to dryness in an oven at 80 °C after which radioactivity was measured by liquid scintillation spectrometry.

Protein estimation. Protein was estimated using bovine serum albumin as standard[20].

Results. Results in the table show that transfer of tritiated methyl groups of SAM (up to 200 μM) was not affected by induction of LLP when assayed under conditions which favor carboxymethylation of proteins. However, when assay conditions which favor methylation of membrane phospholipids were used, the transfer of methyl groups from SAM (< 3 μM) to hippocampal membranes in the majority of experiments was found to be diminished following induction of LLP in slices. At this concentration monomethylation of phosphatidylethanolamine by methylase I (K_m for SAM = 4 μM)[16] is favored. Out of 10 experiments done, 7 showed an average diminution of 19% (table), 1 showed little or no effect and in 2 experiments there was an elevation (about 8%). The reason for the latter effect is unclear at present, but may possibly be due to loss of the LLP effects during the assay procedure. In any event, the methylation process was unaffected with SAM at 200 μM. At this concentration monomethylated phosphatidylethanolamine has been shown to be converted to phosphatidylcholine by methylase II (K_m = 110 μM)[16].

Discussion. It has been pointed out[19,21] that since the cytoplasmic surface of the plasma and vesicular membranes have negative charges, any diminution of this electrostatic barrier could facilitate the exocytotic release of transmitters from the terminals. Protein carboxymethylation has been suggested as one possible mechanism of charge neutralization[19,21]. However, although LLP is accompanied by an enhanced release of glutamine-derived glutamate[7], a fall of tissue glutamate[22], and an enhanced secretion of newly synthesized proteins[8], there is no apparent change in the capacity of hippocampal proteins for carboxymethylation. On the other hand, our results indicate that, with induction of LLP in hippocampal slices, there appears to be a concomitant diminution in the capacity of the membrane phosphatidylethanolamine to be monomethylated (table). It has been shown that incorporation of labelled phosphate from labelled ATP into a specific hippocampal membrane protein is diminished following LLP[13]. This led to the suggestion that increased phosphorylation at the onset of LLP would lead to subsequent diminution in the rate of membrane phosphorylation. By analogy, it may be suggested from our

Effects of induction of LLP in hippocampal slices on the subsequent methylation of proteins and phospholipids

Methylation	% Control concentration of SAM	
	< 3 μM	200 μM
Phospholipid	81 ± 8* (7)	97 ± 4 (5)
Protein	98 ± 6 (4)	96 ± 3 (4)

* $p < 0.05$. (Student's t-test) Number of experiments are given in parentheses. The values are mean ± SD.

work that some of the phosphatidylethanolamine sites on the cytoplasmic side of the hippocampal membrane become methylated at the onset of LLP, so that a subsequent exposure of the membrane to labelled SAM would lead to a diminished incorporation of methyl groups into the membrane. On the other hand, the results may be explicable simply on the basis of a diminished activity of methylase I due to LLP. However, this explanation is less likely since SAM at 200 µM had no effect on phospholipid methylation and, therefore, the successive methylations catalyzed by methylases I and II could not have been suppressed.

Although the exact location is uncertain, there is adequate evidence[2,3,9,24] to indicate that the electrophysiological alterations found in LLP do not occur with stimulated axons or their target somata, but are confined largely to synaptic components. It is, therefore, reasonable to suggest that at least a part of this altered methylation occurs at the synaptic region. In this connection, it is pertinent to note the suggestion that an enhanced rate of phospholipid methylation could account for the effect of SAM in enhancing the rate of spontaneous firing of cerebral cortical neurons[23].

It has been pointed out that methylation of phosphatidylethanolamine and/or the rapid transit of the monomethylated lipid through the membrane decrease membrane viscosity[17]. It, therefore, appears possible that the enhanced membrane fluidity that occurs with phosphatidylethanolamine methylation may partially be responsible for potentiation. On the other hand, if a diminished methylase I activity occured, the resultant enhanced membrane viscosity could explain the decreased excitability of the presynaptic terminal after induction of LLP[9]. An alteration in membrane properties could also account for anatomical changes observed following tetanic stimulations of hippocampal inputs[25–27] which have been implicated in the enhancement of synaptic transmission[26–28].

In conclusion, it is suggested that LLP is associated with an altered membrane viscosity due to an altered rate of methylation of phosphatidylethanolamine. How this change relates to LLP development is at present unclear.

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Relationship between chlorophyll a content and protein content of invertebrate symbioses with algae or chloroplasts

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Summary. The chlorophyll a and protein contents of invertebrates containing autotrophic symbionts are indices of symbiont and host biomass, respectively, and the chlorophyll a content per unit protein is a good measure of symbiont: host ratio. For three different associations, the chlorophyll a content per unit protein is 0.01–0.02 µg µg^{-1}.
Key words. Symbiosis; invertebrate; algae; chloroplasts; chlorophyll a content; protein content.

The size of the population of autotrophic symbionts (unicellular algae or chloroplasts) in association with invertebrates is controlled within narrow limits[2,3]. This is probably achieved through the direct intervention of the host, by digestion or expulsion of excess symbionts or control of symbiont growth rate[2]. Basic to studies of the mechanism(s) by which the host may regulate its symbiont population is a reliable index of the relative size of the partners, i.e. of the 'symbiont:host ratio'. Two indices have been used: the ratio of the biomass of the separate partners and ratio of their volumes. The volume ratio can be estimated from quantitative studies of serial sections[4] or alternatively from the dimensions of the intact association and its symbionts if they approximate to regular shapes, such as spheres or cylinders[2]. Both methods to determine volume ratio are laborious and can be subject to bias and inaccuracy. To determine the biomass ratio, the partners are separated and the biomass of the isolated symbiont and host fractions measured. Although this approach is widely used, it is not satisfactory