glycosaminoglycans, such as those herein identified, are bound to cell membranes of renal tubule cells and hepatocytes[33] and milk fat globule membrane[34].

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Ionic effects on cyclic nucleotide accumulation in a phytophagous insect[1]

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Summary. Incubation in solutions containing high potassium levels reduces specific accumulation of cyclic AMP in larval nerve cords of *Manduca sexta* in vitro with or without serotonin or theophylline.

In previous work by our laboratory on the accumulation of cyclic nucleotides in insect nervous systems[2,3] Grace's tissue culture medium[4] was used as the carrier for incubation solutions. Since this medium contains amino acids which could affect cyclic nucleotide levels, we sought a less complex medium.

The hemolymph of *Manduca sexta* (Lepidoptera: Sphingidae) has a low sodium: potassium ratio, 1:1, which will not support action potential production in naked axons; this implies a sodium-dependent action potential[5]. The ventral nerve cord of *M. sexta* is enveloped by a highly impermeable sheath of glia which allows production on action potentials even in sodium-free saline[5]. Because the freeze-thaw treatment necessitated to obtain pools of nerve cords may have damaged the nerve cord sheath, an investigation of the effects on cyclic adenosine 3′, 5′-monophosphate (cyclic AMP) accumulation of high sodium or high potassium ringer solutions, with or without additions was made.

Materials and methods. Nerve cords from 5th instar 'wanderer' larvae were dissected as previously described[6]. The nerve cords were incubated at 30 °C for 10 min and frozen as described elsewhere[7].

The saline solutions used were a high sodium ringer consisting of 152 mM NaCl, 4.7 mM KCl, and 2.8 mM $CaCl_2$[8], or a high potassium solution, consisting of 150 mM KCl, 5 mM NaCl, and 2.8 mM $CaCl_2$; both were buffered with 50 mM citrate (pH 5.3). Serotonin and theophylline were purchased from Sigma. Ascorbic acid, 100 µM, was routinely added as an anti-oxidant.

Deproteination and cyclic nucleotide extraction was accomplished by homogenizing the frozen solutions in ice-cold absolute ethanol[9]. Cyclic nucleotide residues were assayed by the radioimmunoassay method of Steiner et al.[10] using kits (Collaborative Research, Waltham, MA). Protein was assayed by the method of Lowry et al.[11].

Results and discussion. The data presented in the table indicate that the sodium: potassium ratio has little effect on the resting levels of cyclic AMP in incubated nerve cord pools. Accumulation of cyclic AMP in the presence either of 5-HT or theophylline was markedly higher in the high sodium medium.

These results show that the concentrations of sodium and potassium or their ratio may affect cyclic AMP levels in the *M. sexta* CNS. Because the phosphodiesterase would be essentially unaffected by these conditions[6] we conclude that adenyl cyclase activation is involved.

Adenyl cyclase is variably affected by potassium in mammalian brain slices[12] especially in the presence of theophylline[13,14]. The cockroach, a carnivorous insect with a high Na^+/K^+ hemolymph ratio, accumulates cyclic AMP in the presence of 50 mM potassium with or without theophylline[15]. This report illustrates the difficulties which may be encountered in the extrapolation of results from mammalian to certain kinds of non-mammalian systems.

Specific accumulation of cyclic AMP in pooled nerve cords in saline solutions at pH 5.3

Addition to Ringer solution	High sodium Ringer ($Na^+/K^+ = 32$) (mean±SEM)	High potassium Ringer ($Na^+/K^+ = 0.033$) (mean±range)
None	0.54 ± 0.13 (6)	0.50 ± 0.03 (2)
10 mM theophylline	7.88 ± 2.08 (4)	2.2.75 (1)
10 mM serotonin	5.24 ± 2.65 (4)	1.11 ± 0.09 (2)

Results are given as pmole cyclic AMP/mg protein ± SEM or range (for 2 pools). The number of preparations assayed is indicated in parentheses.

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^{35}S-Sulphate incorporation into myelin sulphated mucopolysaccharides during rat brain development

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Summary. Rat brain myelin acid mucopolysaccharides (AMPS) incorporate 15%, 8%, 5.5% and 4% of tojtal associated ^{35}S-sulphate, 14, 21, 30 and 75 days after birth, respectively. The course of ^{35}S-sulphate incorporation into total rat brain mucopolysaccharides, as well in those from myelin, had a similar feature with peak on the 2nd week and a significant decrease on the 3rd and 4th week postnatally.

The presence of acid mucopolysaccharides (AMPS) in central and peripheral nervous tissue has been demonstrated chemically and histochemically many times[1-3] and it was indicated that these compounds are necessary to maintain normal brain function[4]. Changes in AMPS content in the course of rat brain development and maturation have been reported in a number of papers[5-7]. These macromolecules have been closely studied as mambrane constituents, especially within synaptic plasma membrane, and their possible role in the cell surface regulation of metabolic function has been suggested.

In our previous experiments we identified and quantified hyaluronic acid (HA), heparitin sulphate (HS) and chondroitin sulphates (AéÁC) in myelin isolated from the whole rat brain as well as from different rat brain regions[8-10]. The results obtained induced us to attempt a more precise identification and localization of these compounds in myelin, by studying the incorporation of ^{35}S-sulphate into AMPS extracted from purified rat brain myelin as a function of postnatal development.

White-Hood male rats, 14-, 21-, 30- and 75-day-old, were injected i.p. with 60 µC/100 g b.wt of $Na_2{}^{35}SO_4$ (640 µC/mM) dissolved distilled destilled water and sacrificed by decapitation 16 h later. After decapitation the brains were rapidly removed and weighed; 5% homogenate in 0.32 M sucrose was prepared from whole brain and myelin was isolated and purified according to the method described by Norton and Poduslo[11]. Aliquots from brain homogenate and myelin suspension were used for protein[12] and radioactivity determination (10% Biosolv in a scintillation liquid based on toluol). Water-washed myelin was freeze-dried and weighed.

Purified and lyophilized myelin was delipidated by extraction with chloroform-methanol (2:1) and this was repeated twice. After digestion with N/10 NaOH and neutralization with N/10 HCl, myelin was subjected to proteolysis with activated papain solution[13] at 65 °C for 48 h. Repeated extraction with chloroform-methanol (2:1) and (1:2) were then carried out. After the addition of Lloyd's reagent, the suspension was centrifuged at 2000× g for 15 min. In the supernatant, the remaining proteins were precipitated by 10% trichloracetic acid following centrifugation. Acid mucopolysaccharides contained in supernatant were separated and purified by Ecteola Cellulose microchromatography[13]. After washing the column with 0.025 M NaCl and subsequent elution with 3 M NaCl, the eluate was dialyzed for 48 h. at 4 °C in distilled water by constant stirring. The dialyzed aliquot was used for uronic acid[13] determination, as well as for radioactivity determination[14]. Following lyophilization, individual AMPS were separated by microzone electrophoresis on Cellulose-polyacetate strips in buffered $CuSO_4$ solution (pH 3.4). Individual AMPS wer identified after staining with Alcian blue and subsequent densitometry by comparing with standards running simultaneously (figure 1). Fugure 2 shows that 35% of ^{35}S-sulphate is incorporated into sulphated mucopolysaccharides extracted from the whole rat brain on the 14th day

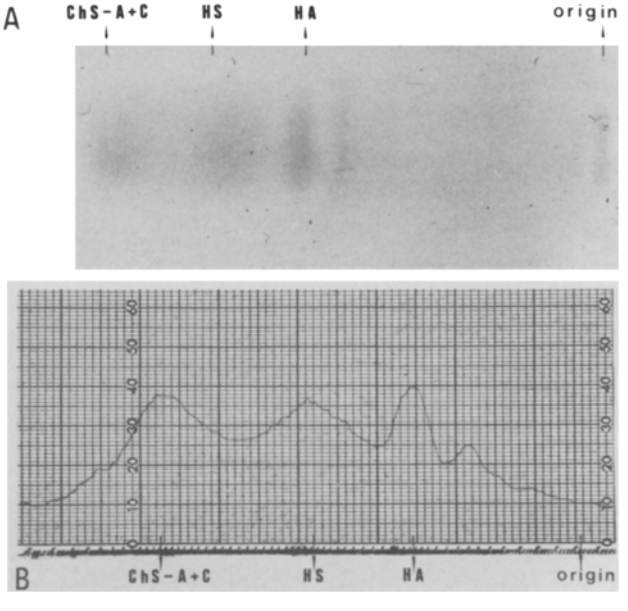

Fig. 1. *A* Electrophoretic profile of individual acid mucopolysaccharides (AMPS) extracted from 14-day-old rats myelin and resolved on cellulose polyacetate strips after staining with Alcian blue. *B* Densitometer scans of individual AMPS resolved electrophoretically and identified by comparing electrophoretic mobility, after staining by Alcian blue, of clearly marked fractions from the analyzed samples, with migration of individual AMPS from standard solutions consisting of highly pure AMPS submitted to electrophoresis simultaneously. Electrophoresis was performed by the method described by Stefanović[17].