AMV-ATPase. Ethanol extraction followed by sonication at an alkaline pH solubilizes the AMV-ATPase[4], while the same treatment completely inactivates the Reo virus enzyme. As the active site of the ATPase in AMV, AMV-infected myeloblast, Rous virus, Reo virus and chicken embryonic cells or fibroblasts is oriented outside to hydrolyse the ATP of the external medium, it may be termed as 'ecto-ATPase'[6,9]. The activity of the enzyme was therefore measured directly by suspending the cells or the virus in the incubating medium. It is worth mentioning that the specific activity of the purified enzyme from AMV is double as compared to the intact virus (tables 2 and 3). It looks as if most of the enzyme molecules in the purified preparation are inactivated. However, the high specific activity of the virus ATPase, the marked loss of activity after solubilization, which could not be restored even in presence of the viral lipids, and also the differential sensitivity of the enzyme towards quercetin (table 2) before and after solubilization, all point to the conclusion that AMV holds the enzyme in a conformation that induces a high ATPase activity of the enzyme. Thus the apparent inactivation may be due to change of conformation after solubilization. Antigenically Reo virus ATPase differs from AMV-ATPase as it originates from different host cells. The immunological cross-reaction between an ecto-ATPase from chicken oviduct and the AMV-ATPase, as reported previously, supports the host origin[4]. The reason why the antibody against the AMV-ATPase did not inhibit the ATPase activity of the chicken fibroblast, or AMV grown on the latter, may be due to masking of the antigenic site in the membrane. Conclusive evidence will emerge from cross-reaction studies after solubilization. It thus appears that Reo virus ATPase is completely different from AMV or Rous virus enzyme as regards their catalytic and antigenic properties. Although Rous virus ATPase behaves similarly to that of the AMV, regarding their sensitivity towards inhibitors, AMV-ATPase has been highly specialized in showing very high catalytic rate not observed in other viruses.

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Adenylate cyclase and phosphodiesterase from brain tissue: Different stabilities during incubation of cerebral cortical slices

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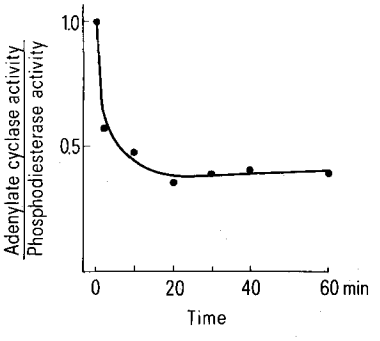
Summary. Adenylate cyclase and phosphodiesterase were prepared from brain slices from guinea-pig. The specific activity of adenylate cyclase declined rapidly with increasing incubation time of tissue slices, while phosphodiesterase activity was almost uneffected by the incubation of brain slices.

Receptor-adenylate cyclase interactions in brain have been studied mainly with slices or homogenates of brain tissue[1]. Both preparations offer distinct advantages, and the resulting data should be viewed together when evaluating the mechanism of hormone elicited cyclic AMP formation. However, no investigations have been reported describing the effect of incubation of brain slices on the enzymic activities of adenylate cyclase (AC) and phosphodiesterase (PDE). In this report, we present data from experiments with cell-free AC and PDE prepared from cerebral cortical slices which had been incubated identical to the usual protocol carried out in studies with brain tissue slices.

Methods. Preparation and incubation of brain cortical slices from guinea-pigs was exactly as described earlier[2]. After various periods of incubation, slices were quickly transferred into a Dounce homogenizer and disrupted in ice-cold buffer containing 48 mM Tris-HCl, 12 mM $MgCl_2$ and 0.1 mM EGTA, pH 7.4. After addition of 5 ml 1 mM $KHCO_3$-solution, the homogenate was centrifuged (5000 × g for 15 min). The precipitate was washed once with $KHCO_3$-solution and the final pellet was dispersed in Tris/$MgCl_2$ buffer. Cyclic AMP formation was determined in a total volume of 200 µl containing 40 mM Tris-HCl, 10 mM $MgCl_2$, 0,1 mM EGTA, 10 µM $CaCl_2$ above EGTA, 1 mM 1-methyl 3-isobutylxanthine (IBMX, EGA-Chemie, Steinheim, BRD), 13,8 mM creatinphosphate, 0.7 mg creatinkinase and 120 µg enzyme protein, pH 7.4. After equilibration at 37 °C, ATP (2 mM) was added to start the reaction. During the incubation, no hydrolysis of newly formed cyclic AMP occurred. After 5 min, the reaction was stopped by addition of 50 mg dry Al_2O_3 and 1 ml 60 mM



Ratio of activities of AC and PDE prepared from cerebral cortical slices which had been incubated for various periods of time (abscissa) in oxygenated Krebs-Ringer-bicarbonate buffer at 37 °C before enzyme preparation.

acetic acid. Cyclic AMP was determined in the supernatant by the binding protein method[3].

When PDE was prepared, the homogenate of the tissue slices was diluted with 8 ml Tris $MgCl_2$ buffer and centrifuged at 15,000 × g for 20 min. PDE activity was assayed in the supernatant with 100 µM cyclic AMP as substrate as described[4]. Activities of adenylate cyclase and phosphodiesterase were linear with protein concentration and with time up to 10 min.

Results. When the particulate AC was prepared immediately after preparation of the brain slices (without incubation in Krebs-Ringer-buffer), enzymic activity was high. Within 20 min of incubation of brain slices, however, AC activity declined to only about one-third and remained at that low level during the residual 40 min of tissue incubation (table). All enzyme preparations could, however, be stimulated significantly by histamine to about the same extent (43±4% stimulation, table).

The capacity of PDE to degrade cyclic AMP in brain is much higher than the synthetic capacity of the AC[5,6]. When PDE was prepared from brain slices incubated for various periods of time in Krebs-Ringer-buffer, the activity/mg protein remained fairly constant at about 90% of the initial value, contrasting sharply the results with AC (table). When the activities of both enzymes prepared immediately after sacrifice of the animal are taken as 100%, the ratio of AC activity over PDE activity is rapidly declining within the first 20 min of incubation of the brain slices (figure), indicating the different stabilities of these enzymes during the tissue incubation.

Activities of cell-free adenylate cyclase and cell-free phosphodiesterase prepared from cerebral cortical slices which had been incubated for various periods of time in Krebs-Ringer-bicarbonate buffer at 37 °C

Incubation time of slices before enzyme preparation (min)	pmoles of cyclic AMP formed per mg protein × min		nmoles of cyclic AMP hydrolyzed per mg protein × min
	+ 100 µM histamine		
0	445±26	594±53	5.8±0.95
2	240±19	320±13	5.5±0.7
10	192±17	263±31	5.4±0.7
20	135±7	189±6	5.2±0.3
30	159±6	239±22	5.3±0.9
40	153±18	240±41	4.9±0.3
60	164±13	252±42	5.4±0.8

Adenylate cyclase activity was determined with identical amounts of protein ± 100 µM histamine, phosphodiesterase was assayed as described[4]. All values are the mean ± SEM of 12 (adenylate cyclase) or 4 (phosphodiesterase) enzyme preparations, assays were performed in duplicates.

Discussion. The results show the significant differences in the stability of AC and PDE during incubations of brain cortical slices. The fact that the receptor-AC interaction seems to be fairly stable, as seen by the uniform stimulation of the cell-free preparation by histamine, is believed to indicate alterations occurring in the enzyme entity. The ratio of enzymic activities of AC and PDE is about 1:100 when the enzymes are prepared immediately after sacrifice of the animal. However, after the usual incubation of brain slices for 60 min[2] this ratio has declined to 1:30. Therefore it seems reasonable to speculate that increases in cyclic AMP concentrations in brain tissue in vivo elicited by neurotransmitters may be much quicker and higher than those obtained in brain tissue slices. This has to be expected if cyclic AMP is to take part in the process of regulation of nervous activity.

The data seem to complicate direct comparisons of results obtained with brain slices and cell-free homogenates, and to emphasize the difficulties in speculating on the mechanisms of regulation of cyclic AMP levels in brain when using cellfree homogenates or tissue slices.

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The absence of gangliosides in a higher plant

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Summary. We report attempts to isolate and purify sialic acid-containing glycolipids (gangliosides) from etiolated hypocotyls of soybean *(Glycine max)* using methods developed for rat liver. The maximum amounts of ganglioside sialic acid present was found to be less than 0.021 nmoles/g fresh weight or less than 1:100,000 the amounts present in rat liver. We conclude that this tissue lacks gangliosides.

Gangliosides are negatively charged glycosphingolipids that contain oligosaccharides composed of glucose, galactose, and N-acetylglucosamine and 1 or more terminal sialic acid (N-acetylneuraminic acid) residues. Sialated glycosphingolipids (gangliosides) are found in echinoderms and vertebrates, particularly concentrated in nervous tissue of the latter[1]. The presence of gangliosides in higher plants has not been investigated nor has the presence of sialic acid been shown convincingly. Cabezas[2] disputes early claims for the existence of sialic acid in plants based on the discovery of several materials in extracts of plants that interfere with the thiobarbituric acid assay used to detect sialic acid. Gielen[3] earlier identified a 2-keto-3-deoxyaldonic acid in plant material capable of yielding a positive reaction in the assay. However, the occurrence of glycoproteins[4,5] and glycolipids[6] in plants is accepted widely. Of particular interest was the report by Bailey[7] of the occurrence of cerebrosides, the immediate, asialo precursors of gangliosides, in chloroform extracts of mung bean *(Phaseolus aureus)* hypocotyls. We report here an attempt to isolate and purify gangliosides from hypocotyls of soybean.

Materials and methods. Ganglioside purification was ac-