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-

cording to Ledeen[8]. Soybeans *(Glycine max* L. cv Wayne) were grown for 4 days in darkness as described[9]. Intact hypocotyls (ca 500 g) from which the cotyledons were removed were homogenized in chloroform:methanol (2:1 v/v) using a Polytron 20ST homogenizer (Kinematica, Lucerne, Switzerland) and diluted to 2000 ml with the same chloroform:methanol mixture. The resulting homogenate was stirred for 3 h at 10 °C and filtered. The filtrate was extracted with an additional 2000 ml chloroform:methanol (1:1 v/v), stirred overnight, and filtered. The combined extracts were concentrated to a thick paste by rotary evaporation (50 °C), resuspended in 100 ml chloroform:-methanol:water (60:30:8 v/v) (solvent A), and applied (1-2 ml/min) to a DEAE sephadex column[8]; the column was washed with an additional 600 ml of solvent A (3-5 ml/min), and the acidic lipids eluted with 2000 ml of methanol:chloroform:0.8 M sodium acetate (60:30:8 v/v) and evaporated to dryness. The residue was resuspended in 100 ml 0.1 N methanolic sodium hydroxide, with stirring, saponified at 40 °C for 2 h, and lyophylized. This residue was resuspended in chloroform:methanol (4:1 v/v), filtered to remove insoluble material, and applied to a Unisil column[8]. The column was eluted first with 1000 ml chloroform:methanol (4:1 v/v) to remove contaminating lipids and then with 1000 ml chloroform:methanol (1:1). This eluate was concentrated to 10 ml and stored at −20 °C. Sialic acid was analyzed spectrophotometrically by the thiobarbituric acid method of Warren[10]. Additionally, the samples were chromatographed along with known amounts of authentic ganglioside G_{M3}[11] on silica gel G thin layer plates (Analtech, Inc., Newark, Del.) using chloroform:methanol:2.5 N ammonium hydroxide (60:40:9 v/v) as solvent. Lipids were visualized with iodine vapors and sialic acid was visualized using a resorcinol reagent[12].

Results and discussion. Analysis of the presumptive ganglioside fraction using the thiobarbituric acid assay[10] failed to detect sialic acid in each of 3 replicate experiments. Under ideal conditions, the assay will detect sialic acid amounts as low as 5 nmoles. Values for the maximum amounts of ganglioside sialic acid that might be present and still not be detected by this assay method would be on the order of 0.085 nmoles/g fresh wt of hypocotyl tissue. Normal values for rat liver are 2,600 nmoles/g fresh wt. Similarly, analyses of the soybean concentrate by TLC showed no compounds migrating with authentic G_{M3}, the simplest of the monosialogangliosides, and no compounds giving a positive reaction with resorcinol reagent[12] for sialic acid. Using this latter method, we were able to detect as little as 0.04 nmoles G_{M3} sialic acid. Based on this value, the maximum amounts of ganglioside sialic acid in the soybean hypocotyl tissue would be less than 0.02 nmoles/g fresh wt or less than 1/100,000 the amounts present in rat liver. Thus, we conclude that gangliosides, for all practical purposes, are absent from etiolated hypocotyls of the soybean.

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Estimation of the methylating capacity in the pineal gland of the rat with special reference to the methylation of N-acetylserotonin and 5-hydroxytryptophol separately

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Summary. In the present paper, an extension is presented of an earlier described method, by which the methylating capacity of the pineal gland can be determined. Supplementary to the earlier method, the synthesis of melatonin and 5-methoxytryptophol can now be qualified and quantified separately.

Recently Balemans et al.[2] described a method by which the methylating capacity of the pineal gland for several 5-hydroxyindoles and norepinephrine can be determined. However, in this method melatonin and 5-methoxytryptophol could not be determined separately. As melatonin and 5-methoxytryptophol are probably the most important indoles in the pineal-gonadal correlation[3-6], it remained necessary to determine the methylation of both 5-methoxyindoles separately. Therefore an additional method was developed.

The procedure was as follows: 1 rat pineal was slightly disrupted and incubated in 20 µl 0.1 M of phosphate buffer pH 8.0 and 10 µl of (^{3}H)-S-adenosyl methionine (containing 0.7 µCi in H_2SO_4 pH 2.5) for 60 min at 37 °C. The 5-hydroxyindoles present in the pineal were used as a substrate which implies that no substrate was added. After stopping the reaction with 10 µl H_2SO_4 pH 1.0, the pineal tissue was homogenized and synthetic 5-methoxyindoles were added for reference purposes. The pineal tissue together with the incubation medium was then chromatographed by TLC (Merck DC-Silicagel plates 60F 254, 025 mm No. 5729 were used). To minimize decomposition, chromatograms were developed in darkness. A bidimensional TLC-technique was applied. As a solvent system for the 1st direction, chloroform:methanol:acetic acid (93:4:3) was used. The plates were dried under nitrogen and developed in the 2nd direction in chloroform:methanol:ammonia 25% (60:35:5). After chromatography in the 1st direction S-adenosyl methionine, 5-methoxytryptophan, 5-methoxytryptamine and normetanephrine remain on the start. The R_f values of melatonin, 5-methoxytryptophol and 5-methoxyindole-3-acetic acid are respectively 34, 51 and 50. Thus in this solvent system 5-methoxytryptophol could not be separated from 5-methoxyindole-3-acetic acid. After turning the thin layer plate 90° and application of the 2nd solvent system, 5-methoxytryptophol and 5-methoxyindole-