As for the frequency bands, the mean values and SEM were quantified for factor 1 in RMS μV and integrated over the experimental time (figure 6A). The time integral in rabbits receiving DSIP was compared to that in control rabbits. For factor 1 in the neocortex, the difference between integral increase in DSIP rabbits and decrease in controls reached 53.3 ± 10.7% (figure 6A). The statistical significance of factor 1 was calculated with the same nonparametric test as for the frequency bands. The significance of factor 1 increase in neocortex was above 90% ($p<0.01$), with biorhythmic maxima up to 99.9% (figure 6B). In the limbic cortex, for factor 1, the difference was higher than in the neocortex: 78.7 ± 15%. The significance reached a maximum value of 99.9%, without marked biorhythmic oscillations.

Discussion and conclusions. The original nonapeptide (mol.wt 848.98), identified in the last fraction of cerebral blood dialysate from thalamus stimulated rabbit donors, induces, besides bradypnea and bradycardia, a delta + spindle EEG sleep, when infused into the mesencephalic ventricle of rabbit recipients. Because of its significant delta EEG enhancing activity (quantified in RMS μV and time integral), this compound was called original 'delta sleep inducing peptide', DSIP. The peptide level in plasma of awakened rabbits (0.05 mg) is 9 times lower than that of rabbits submitted to stimulation of the somnogenic thalamic area (0.47 mg).

Synthetic DSIP induces a similar activity in the delta and spindle frequency bands of the rabbit's neocortex, with ultradian biorhythms of 3–6 cph. The delta + spindle activity is even higher in the limbic cortex. The specificity of the original and synthetic DSIP is established by comparing their delta activity with that of peptide analogues; the latter were found to be inactive.

The original and synthetic DSIP have the same effective dose (~ 6 nmoles/kg intraventricularly infused in rabbit). Definite features suggest that DSIP might act as a programming modulator at supra-operational level rather than as a transmitter at operational level (long latency, activation of latent biorhythmic oscillators, reversibility of the EEG sleep effect under influence of waking stimuli). DSIP might pass the blood-brain barrier, since ultrafiltration through UM-05 filters is possible for peptides with mol.wt above 1000 or bacitracin with mol.wt = 1400. This is also supported by the fact that i.v. injection of synthetic DSIP in free moving rabbits induces an EEG delta activity up to 144% during 5 h following a reference period of 90 min against 126% in control rabbits (unpublished data). Concurrently the motor activity decreases. DSIP differs by its higher mol.wt from factor S of Pappenheimer et al.[20], extracted from the goat's csf or sheep's brain, and from the sleep-promoting material of Uchizono and coworkers[21] extracted from the rat's brain. It furthermore differs from factor S by the fact that, in the intraventricular tests in rabbits, the EEG delta effects are detectable already during the infusion period and without concomitant 'epileptiform episodes'.

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PRO EXPERIMENTIS

A method for demonstrating zinc content of the brain using 2-carboxy-2′-hydroxy-5′-sulfoformazylbenzene perfusion-staining

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Summary. A rapid accurate method for histochemical localization of zinc in the rat brain, utilizing 2-carboxy-2′-hydroxy-5′-sulfoformazylbenzene perfusion-staining, is described.

Zinc is an essential element in animal nutrition; deficiency or intoxication produce characteristic symptoms. This element forms an integral part of a number of metalloenzymes such as carbonic anhydrase, alkaline phosphatase, lactic dehydrogenase and alcohol dehydrogenase[2]. The dithizone method is commonly used for histochemical demonstration of zinc[3], but dithizone (diphenylthiocarbazone) forms an insoluble, coloured inner complex salt with a number of heavy metals (Zn, Pb, Ag, Cu, Hg, Au, Cd). Zincon (2-carboxy-2′-hydroxy-5′-sulfoformazylbenzene, figure 1) has recently been used for serum zinc determinations[4]. Under carefully controlled conditions, according to Searcy[4], this procedure yields results with an acceptable degree of precision and accuracy. Furthermore, Zincon has been successfully used as an indicator for the spectrophotometric determination of the zinc content of water[5].

Materials and methods. Preparation of experimental animals. A total of 35 male albino rats weighing 100–150 g were used for this study. I. p. injections of 5 mg elemental zinc per kg b.wt in the form of zinc chloride were given daily for 5–7 days. Atomic absorption spectrophotometric estimation of the level of zinc in different regions of the brain of 20 rats (10 zinc-treated and 10 control rats receiving equal volumes of normal saline) was carried out using a Perkin-Elmer model 303 atomic absorption spectrophotometer. Details of this experiment form part of a separate communication[6].

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Preparation of solutions for perfusion staining. 1. Staining solution. Dissolve 0.130 g of 2-carboxy-2′-hydroxy-5′-sulfoformazylbenzene (Zincon, cat No. 3750, Sigma Chemical Co., St. Louis, USA) in 2 ml 1 N NaOH and dilute to 100 ml with redistilled water. 2. Buffer. Dilute 213 ml 1 N NaOH to 600 ml with redistilled water. Dissolve 37.3 g KCl and 31.0 g H_3BO_3 in the solution and dilute to 1:1 with redistilled water. 3. Perfusion fluid. To 100 ml of solution 1 add 200 ml solution 2 (or enough to bring pH to 10.0).

Procedure for perfusion staining. Rats were deeply anesthetized with pentobarbital sodium and, after opening the thoracic cavity, a polyethelene cannula was inserted into the ascending aorta through the left ventricle. The rate of perfusion was adjusted to 8 ml/min. The right auricle was opened to allow a low-resistance return of the perfusion fluid from the head. The perfusion was discontinued after 10–15 min when oozing of the fluid was apparent from a cut made in the upper lip. The skull was opened, the brain removed carefully and immediately frozen on the freezing stage of a cold microtome (Kryotome Model 1310 K, Leitz, Fed. Rep. of Germany). Frozen sections 20 µm thick were obtained after proper trimming of the block. They were collected on glass slides and dried. Some of the frozen sections were further stained with the same buffered staining solution (pH 10) for 0.5 min, then rinsed with deionized water. Glycerol gelatin (Catalogue No. GG 1, Sigma Chemical Co., St. Louis, USA) was used as the mounting medium.

Microscopy and photomicrography. Sections were examined with an Orthoplan universal large field microscope (E. Leitz, Wetzlar, Fed. Rep. of Germany) and the photomicrographs of the appropriate regions (figure 2) were obtained using an Orthomat W automatic microscope camera (E. Leitz).

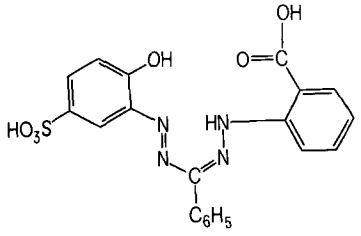

Fig. 1. The structural formula of 2-carboxy-2′-hydroxy-5′-sulfoformazylbenzene (Zincon).

Fig. 2. Photomicrograph of a frozen section of rat cerebrum showing zinc-Zincon reaction product in the form of dark blue granules and radiating fibres. Bar = 50 µm.

Results and discussion. The Zincon staining solution, which is deep red, forms a blue complex with zinc ions, especially in alkaline solutions (pH 9–10). Cold knife or cold microtome sections are undoubtedly best for the demonstration of natural tissue zinc. The zinc complex is stable over a pH range of 8.5–10, whereas the copper-Zincon complex is stable[5] in the pH range 5.0–9.5. This difference in effect of pH permits the ready detection of zinc at pH 9.5–pH 10. The dithizone method which is commonly used for histochemical demonstration of zinc is not so specific. The reddish dithizone staining solution forms a reddish-purple reaction complex with not only zinc but with a number of other heavy metals (Pb, Ag, Cu, Hg, Au, Cd) as well[3]. Atomic absorption spectrophotometric analysis of the brains obtained from the same group of rats has revealed that the Zincon method was highly sensitive to as low a concentration as 20 µg of zinc/g brain tissue. A unique characteristic of Zincon is its ability to diffuse uniformly to almost every part of the brain to react with zinc intravitally. The method described in this paper can be advantageously used to localize minute amounts of zinc rapidly and accurately.

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Intracellular pH of Limulus ventral photoreceptor measured with a double-barrelled pH microelectrode[1]

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Summary. The intracellular pH of the Limulus ventral photoreceptor was measured with a double-barrelled pH microelectrode and found to be 7.01 ± 0.04 SE (n = 9).

In the course of studies on the light transduction mechanisms in Limulus ventral eye, experiments have been reported in which calcium buffers[3], pH buffers[3,4] and calcium sensitive dye[5] have been injected into the photoreceptor cells. Since the properties of these injected substances depend on pH, it is particularly interesting to have a value for the intracellular pH of these cells.

Materials and methods. The double-barrelled pH microelectrodes were made by a modification of the technique developed by Puccaco and Carter[6]. The principle consists of sealing a thin membrane of pH-sensitive glass over the tip of a micropipette made of a high electrical resistance, pH-insensitive glass. Although the technique worked well for single-barrelled pH microelectrodes, there was some