Zusammenfassung. Bei elektrophysiologischer und biochemischer Funktionsprüfung des Bauchmarkes von *Periplaneta americana* wird gezeigt, dass die frasshemmenden Chinone Juglon und 1,4-Naphthochinon sowie N-Aethylmaleimid sowohl die endogene Nervenaktivität wie auch ATPase hemmen, während Menadion nur auf die Nerven wirkte. 2-OH-1,4-Naphthochinon und *p*-Chloromercuribenzoat hemmten nur die ATPase und Iodacetamide hatte keinerlei Effekt.

J.E. BAKER and D.M. NORRIS

Department of Entomology, University of Wisconsin, Madison (Wisconsin 53706, USA), 12 July 1971.

Some Physical-Chemical Properties of the Rabbit's «Sleep Hemodialysate»

We have previously described an improved method for producing sleep hemodialysate, free of waking factors, which provided new evidence of humoral transmission of sleep[1,2]. We now attempt a preliminary physical-chemical characterization of the 'sleep' dialysate in view of obtaining more information on the so far hypothetic, hypnogenic factor or factors responsible for humoral transmission of sleep in our experimental model.

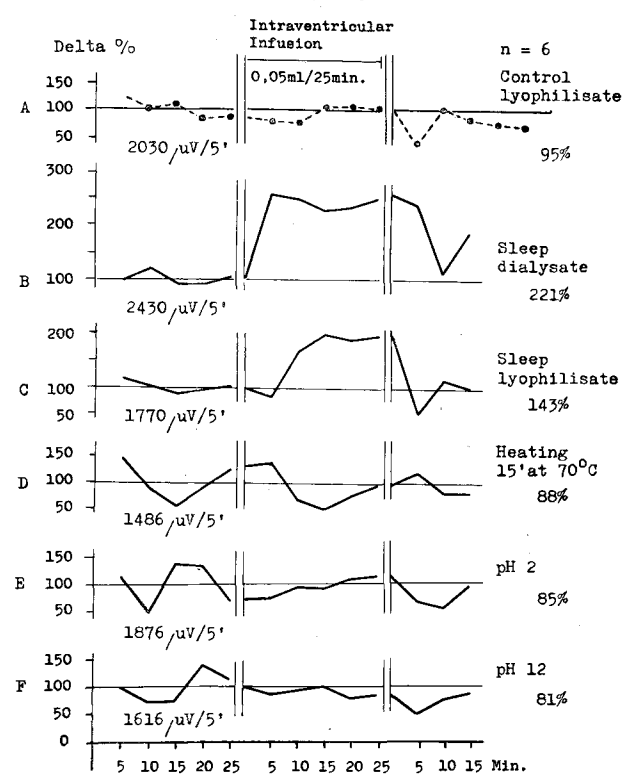
Methods and material. a) *Production of sleep and of sleep hemodialysate.* During extracorporal hemodialysis, experimental sleep is induced in rabbits by electrical stimulation of the ventro-medical intralaminary thalamus[1,2]. Control animals are submitted to sham-stimulation of the same region ,i.e. with electrode in place, but without stimulating current. Sleep is expressed by changes in the electrical brain activities recorded with an electroencephalograph of Schwarzer and submitted to an automatic frequency analysis (Faraday Instr., London). The best quantitative criterion of ordinary sleep is the increase in *amount of slow delta activities* in the motor cortex[1,2]. During a preliminary dialysis of 30 min, the simultaneously recorded average delta activity is used as reference for quantification of the increase in 'sleep' occurring during the following chief dialysis and stimulation period of 60 min. The average delta value of the pre-dialysis per 5 min is chosen as reference = 100%, for example 170 mm deflection corresponding to 2030 μV delta activities. Sleep is expressed by a significant increase, and arousal by a decrease of these delta activities.

b) *Permeability of the dialyzer membranes.* During dialysis, venous blood from the brain flows in the internal channel, whereas a protein-free 'serum-like' dialyzing fluid flows countercurrent in the outer channel of the dialyzer. In all dialysis experiments, cellophane membranes with 25–30 Å pore size are used. The membranes were found to be impermeable to ovalbumine(M.W. 44.000), but permeable to myoglobin (M.W. 17.800). Under these conditions, only blood constituents of low molecular weight must be assumed to appear in the dialysate.

c) *Assessment of the dialysate's hypnogenic activity.* The hypnogenic effect of fresh or lyophilized sleep dialysates is tested by infusion into the meso-diencephalic ventricular system of rabbit recipients[3]. The electrographic delta activities of the mildly restrained animals, previously shown to vary in close correlation with the motor activity of the free-moving animal[2] are considered here as a fully reliable parameter of the hypnogenic effect.

The *osmolarity* of the dialysates is controlled by measuring the freezing point with the osmometer of Knauer and expressed in milliosmols (courtesy of Prof. H. REBER). Simultaneously *conductivity* measurements are carried out, using a conductivity-meter (Radiometer Ltd, Copenhagen/Denmark). This careful control before infusion excludes hypnogenic test artefacts due to differences in concentration of low molecular weight solutes, such as transmembraneous concentration gradients alone or subsequent changes of osmotic properties.

We investigated the influence of storage, freezing and thawing, multiple freeze-drying, heating and extreme pH changes upon the hypnogenic activity of sleep dialysate. The results reported here were obtained from a series of 13 fresh sleep dialysates, 5 sleep lyophilisates and 6 control dialysates. They are summarized on the Figure which reproduces, as an example, the effects of various influences on the same dialysate.



Effects of various physical-chemical conditions on the hypnogenic activity of sleep dialysate tested by intraventricular infusion and EEG recording of delta activities. A: Lyophilisate from 6 control donors. B–F: Example of lyophilisate from the same donor, submitted to different conditions.

1 M. MONNIER and A. M. HATT, Experientia *27*, 722 (1971).
2 M. MONNIER and A. M. HATT, Pflügers Arch. *329*, 231 (1971).
3 M. MONNIER and A. M. HATT, Pflügers Arch. *317*, 268 (1970).

Results. – Osmolarity and pH. Sleep dialysates have an *average molarity* of 309 ± 1 mOsm (milliosmol) and a corresponding *conductivity* of 13.5 ± 1.5 mS (millisiemens), i.e. values similar to those of cerebrospinal fluid. They may therefore be infused into the cerebral ventricular system without disturbing the equilibrium between the ventricular and periventricular space.

During dialysis, the blood pH (measured with an E_A 520 electrode equipped with a corresponding compensator E 388; Metrohm Ltd, Herisau, Switzerland) of the cerebral venous blood flowing through the inner channel of the dialyzer decreases from 7.41 to 7.37 during a dialysis time of 60 min, a decrease most likely due to increased acid metabolites. By contrast, the pH of the dialysates from sleeping or control donors shows an increase from 7.39 to 7.50, due to a partial loss of CO_2 which can be prevented by administration of oxycarbon during the dialysis. However, at room temperature, the dialysate's pH increases again and must be corrected by phosphate buffer for intravenous injection (7.38) or intraventricular infusion (pH 7.24) to recipient rabbits. It must be emphasized that these minor pH changes occur both in sleep and control dialysates; this fact and the pH readjustment performed before injection or infusion exclude any possible influence of pH differences simulating a dialysate's sleep effect.

Storage, freezing and thawing; lyophilization. The hypnogenic effect of the hemodialysate slightly decreases after storage over a period of 2 days at 4°C. Furthermore, by freezing and thawing after three weeks storage time, the dialysate loses more than 50% of its hypnogenic effect. In order to overcome this loss, the dialysates must be immediately freeze-dried and the solid residue stored at −20°C. However, even after ordinary *lyophilization*, there is a slight loss of the hypnogenic delta activity, as shown in a first experiment (Figure B, C.). Thus, the delta activity, amounting to 221% in recipient rabbits receiving an intraventricular infusion of fresh sleep dialysate, decreases to 143% in those receiving sleep lyophilisate, redissolved with metal free distilled water. This fact suggests that a highly labile compound is probably responsible for humoral transmission of sleep.

Heating. In a second set of experiments, boiling of the sleep lyophilisate (15 min at 100°C) in a water bath, completely abolishes the sleep inducing activity. Even a milder treatment of the hypnogenic dialysate (warming up of 2 ml to 70°C for 15 min in water bath, followed by cooling) completely suppresses the hypnogenic effect; indeed, the amount of delta activities no longer increases, but drops to 88% against 143% for the untreated lyophilisate (Figure D). This thermolability confirms our previous assumption.

Extreme pH changes. In a third series of experiments, 2.5 ml sleep lyophilisate are submitted to *acidification* and brought to pH 2 by HCl; final concentration 0.151 M HCl. After 1 h, the mixture is neutralized to pH 7.38 with NaOH. An increase of 0.00177 g NaCl/ml results from this procedure, which was compensated for in the corresponding «control» dialysate. Here again, after final pH adjustment at 7.24, the intraventricular infusion of this dialysate no longer increases (85%) the delta activity of sleep in recipient animals, as shown in Figure E.

The hypnogenic activity is likewise completely abolished by *alcalization* of 2.5 ml sleep lyophilisate to pH 12, followed by neutralization after 1 h in the same way as described above (delta amount 81%. Figure F). We may therefore conclude that the hypnogenic property of sleep dialysate is also extremely susceptible to great changes in pH.

Discussion. The hypnogenic activity of rabbit's sleep dialysate, under controlled conditions of pH and osmolarity, seems to be related to one or more compounds present in the dialysate. These compounds lose part of their activity by freezing and thawing, also by lyophilization of the fresh sleep dialysate, as already shown by MONNIER and HATT[2]. The active compounds are also altered by storage over a long period of time at 4°C. They cannot be proteins of high molecular weight, since these could not pass through the dialyzer membrane. For the same reason, hypnogenic artefacts due to bacterial contamination during long storage may be excluded. Moreover, the extreme heat instability and susceptibility of the active components to changes of the pH strongly suggest that the hypnogenic activity of sleep dialysate might be due to specific low molecular weight organic substances.

Zusammenfassung. Die hypnogene Aktivität des Schlafdialysates von Kaninchen hängt nicht von geringen Änderungen des pH, sondern von einer oder mehrerer Verbindungen des Dialysates ab. Diese Verbindungen verlieren anscheinend einen Teil ihrer Aktivität durch längeres Lagern im Kühlschrank, wiederholtes Einfrieren und Tauen sowie durch Lyophilisieren des frischen Dialysates. Sie sind keine Proteine höheren Molekulargewichtes, da solche die Membranen des Dialysiergerätes nicht passieren können. Schliesslich spricht die Empfindlichkeit der aktiven Verbindungen auf Hitze und extreme pH-Änderungen für eine Beziehung der hypnogenen Aktivität des Schlafdialysates zu spezifischen organischen Verbindungen von niedrigem Molekulargewicht.

M. MONNIER[4] and G. A. SCHOENENBERGER[5]

Physiological Institute, University of Basel (Switzerland), 15 June 1971[6].

[4] Head of the Physiological Institute.

[5] Head of the Biological-Chemical Research Division, Department of Surgery, Basel Medical School.

[6] Report at the «Max Planck Institut für experimentelle Medizin», Göttingen (Department of Prof. W. VOGT).

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Serial Reconstruction with the Electron Microscope of Carotid Body Tissue. The Type I Cell Nerve Supply

The carotid body is a structure which lies at the bifurcation of the carotid artery and samples the arterial blood passing to the head. Within it is a chemoreceptor which senses the concentrations of oxygen, carbon dioxide and hydrogen ion in the blood. Information concerning these chemicals is relayed through the sinus nerve to the brain. The traditional view of this structure is that the sensor is a cell, the Type I cell, and information passes