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.

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

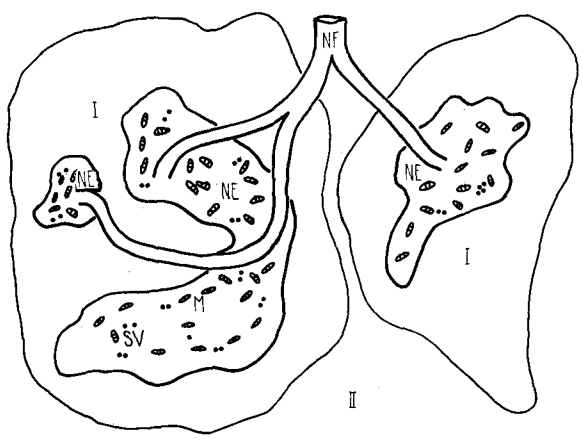
The carotid body is a structure which lies at the bifurcation of the carotid artery and samples the arterial blood passing to the head. Within it is a chemoreceptor which senses the concentrations of oxygen, carbon dioxide and hydrogen ion in the blood. Information concerning these chemicals is relayed 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

from this cell to a nearby nerve ending; both Type I cell and nerve terminal are enclosed by a Type II cell. Doubt has been cast upon this interpretation of the structure by the recent experiments of BISCOE, LALL and SAMPSON[1]. The more recent interpretation of structure elaborated by BISCOE[2] is that the Type I cell is not the sensor but part of an efferent system controlling the sensitivity of the sensor. The nerve ending adjacent to the Type I cell is an efferent nerve ending and the sensory ending is to be found elsewhere. Two questions arise concerning this hypothesis 1. What is the strucuture of this efferent system ? 2. Which nervous structure is the receptor ?. In an attempt to answer these questions we have undertaken a serial reconstruction of carotid body tissue using the electron microscope. By this means the efferent system should be fully defined and the sensory nerve termination identified. (In the fully developed hypothesis it is proposed that the alternative nerve termination is non-myelinated, is very small in diameter (approx. 0.1 μm), is less than 100 μm long, and is surrounded by the Type II cell.) So far we have been able to elucidate the nerve supply to the Type I cell and that topic is the subject of this report.

The study was made on gluteraldehyde fixed, cat carotid body tissue embedded in Epon (see BISCOE, LALL and SAMPSON[1] for details). The material was cut with a diamond knife in series of 300–400 sections approximately 700×10^{-10} m thick. The sections were mounted in groups varying between 7 and 20 sections on formvar films covering single slot grids, stained in uranyl acetate and lead citrate, and viewed and photographed with a Siemens Elmskop I microscope at 80 kV. A cell was selected for study in the middle of the series and photographed. All sections were then photographed on either side of this mid-point until the cell had been completely included in the photographic series. This procedure has been carried to completion on 3 Type I cells and partially so on several others. Since it is obviously impossible to publish all or even a substantial part of our evidence in a short communication we have deemed it best to make a drawing which embodies all our findings in so far as they relate to the Type I cells and the synaptic vesicle containing endings associated with them (Figure). Each Type I cell has applied to its surface 1 to 3 nerve endings of variable size, 1 at least of which may be very large, rather like a very irregular saucer, plate or disc, applied to the surface of a football. These nerve endings are supplied by branches of the same nerve fibre, non-myelinated at this point. The nerve fibre may also supply at least one and possibly all of the remaining Type I cells in a Type I/Type II cell group. The ratio of cell types in such a group will be 4–5/1. Serial section through these nerve endings may show all of the features so far described in the literature. The occurence of various combinations of these features in sections of nerve endings has led to the assertion by some authors that more than one type of nerve ending is to be found on a Type I cell (see BISCOE[2]). This is not the case in our series and there is no evidence to support this claim. That is to say the same nerve ending may show areas of few or areas of many synaptic vesicles; may be applied over a large area, though section in another plane would suggest a small ending; may have areas where there are many mitochondria and where there are few; will always show intermittent electron dense junctional regions with the Type I cell; may have a nerve which also supplies other smaller endings on the self-same Type I cell.

These results suggest that the Type I cells along with their nerve endings are connected by nerve fibres into a unit. If these nerve endings are indeed efferent then the structures form a motor unit. We have yet to show that all the Type I cells in a group are supplied by the same nerve, though this seems probable, and it is possible that Type I cells in adjacent groups may also receive innervation from the same nerve. We have yet to seek a correlation of structure with the nature of the Type I cell amine content. These problems together with that of receptor termination will only be resolved by reconstruction of a complete Type I/Type II cell complex which we are undertaking.

What does seem quite clear, and which has not been previously established, is that there is only one nerve supplying the nerve endings on a Type I cell and that the nerve endings are morphologically of the same type though differing in size and shape. Thus section in different planes may demonstrate differing aspects of the structure of these nerve endings.



The drawing embodies our findings from serial section studies of the Type I cell in the carotid body of the cat. A nerve fibre, non-myelinated at this point, branches to supply nerve endings on adjacent Type I cells. There is usually more than one nerve ending on each Type I cell. The nerve endings are always of the same type and contain small mitochondria, synaptic vesicles and have electron dense junctional regions with the Type I cell. The whole structure is enveloped in Type II cell whose membranes are not shown for the sake of simplicity. I, Type I cell; II, Type II cell; NE, nerve ending; NF, nerve fibre, M, mitochondrion; SV, synaptic vesicles.

Zusammenfassung. Rekonstruktionen aus elektronenmikroskopischen Serienschnitten ergeben, dass einige Zellen von Typ I im Glomus caroticum durch eine efferente Nervenfaser zu einem Komplex zusammengefasst werden. Underschiede in den Synapsen an der Oberfläche der Typ I-Zellen wurden nicht gefunden.

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