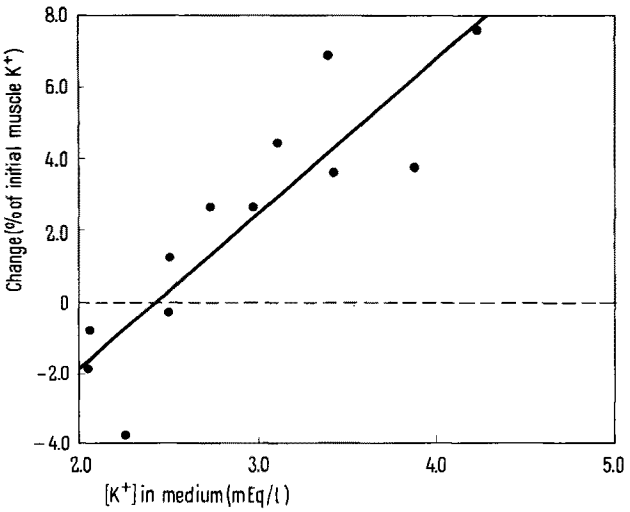
after incubation at 20°C for 6 h is shown as a function of the potassium content of the Ringer's solution (Figure). The calculated regression line drawn in the Figure is given by the equation $y = -10.3 + 4.25\,x$ and the regression coefficient, 4.25, is highly significant ($P < 0.001$). The line passes through the zero change point (shown by the broken line) at a potassium concentration of 2.44 mEq/l, indicating that the fluxes of potassium into and out of the sartorius muscle are equal when the muscle is bathed at 20°C in Ringer's solution containing this concentration of potassium and 5 mM sodium lactate.



The effect of the concentration of potassium in Ringer's solution on the net change in the potassium content of sartorius muscle from *R. pipiens*. NaCl was replaced by KCl so that the sum of Na + K remained constant in each different Ringer's solution. The average weight of each muscle was 98 mg. Zero change in muscle potassium is indicated by the broken line. Each point is the average of determinations in at least 4 muscles. The solid line is the calculated regression line (see text).

It is clear, therefore, that under identical *in vitro* conditions the paired tibialis anticus and iliofibularis muscles and the sartorius muscle require different extracellular concentrations of potassium to maintain a steady state with respect to intracellular potassium. Since the normal concentration of potassium in the plasma presumably provides a steady state for the potassium content of all of these muscles *in vivo*, this *in vitro* phenomenon must be related to the peculiarities of the experimental conditions and to the differences in the size and shape of the muscles. Thus it is concluded that when steady state conditions are important, the concentration of potassium in Ringer's solution must be adjusted to fit the particular experimental conditions and the specific muscles under study.

Résumé. Pour être certain que le dégagement et l'absorption du potassium sont exactement compensés dans un muscle de grenouille étudié *in vitro* dans la solution de Ringer, il faut adapter la concentration du potassium de cette solution aux conditions particulières de l'expérience et tenir compte du fait que le degré de concentration requis varie d'un muscle à l'autre.

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Threshold for Eliciting the Hippocampal theta-Rhythm by Electrical Stimulation in Tegmentum and Hypothalamus during Cortical Spreading Depression in Rats

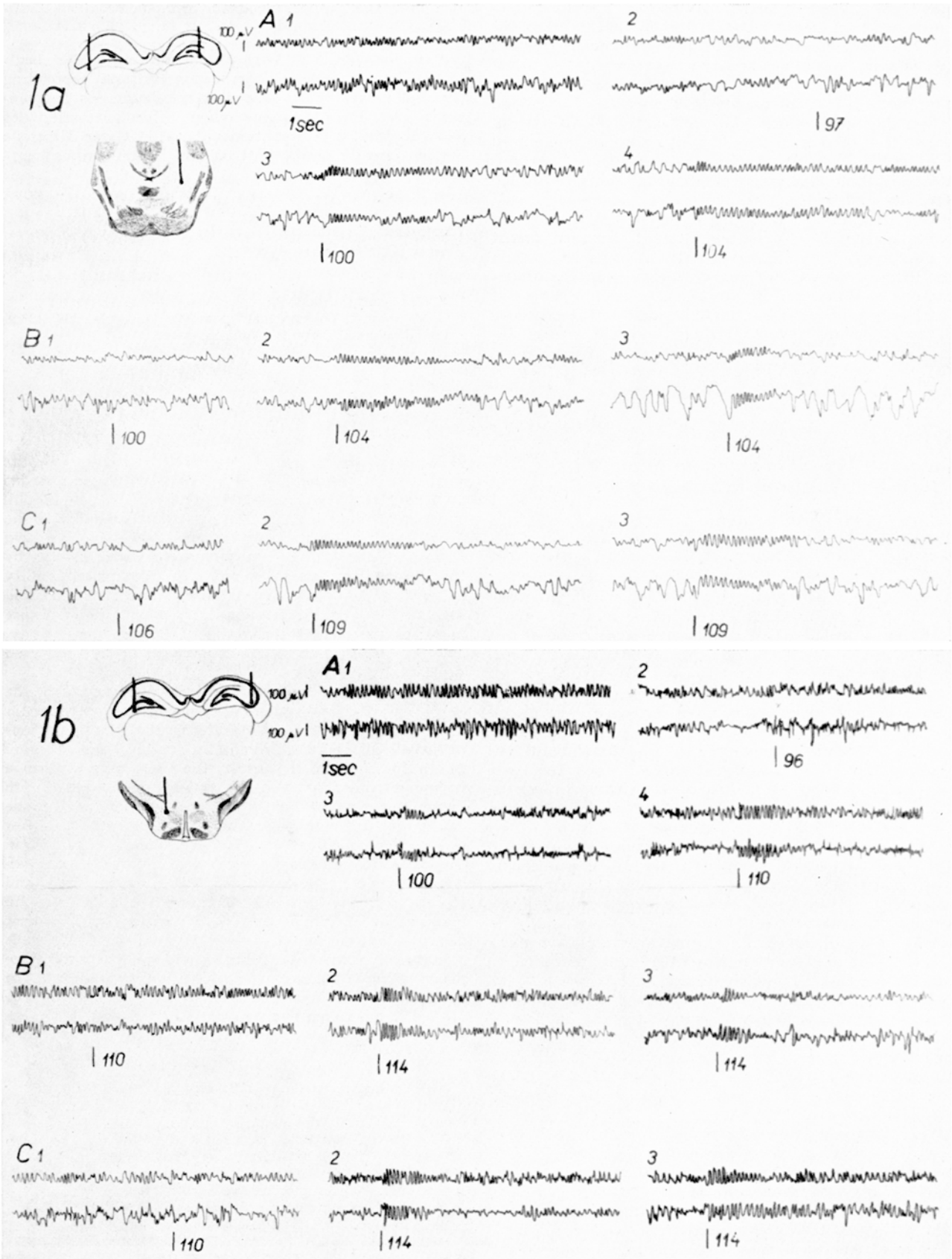
Electrical stimulation of the mesencephalic reticular formation elicits a synchronized theta-rhythm in the hippocampus (GREEN[1]). Since this reaction is uninfluenced by bilateral cortical spreading depression (SD) (WEISS and FIFKOVÁ[2]), it is possible to use the hippocampal arousal reaction as an indicator of the excitability of the tegmental reticular formation (RFT) and of the hypothalamus (Hy) during reversible elimination of the cerebral cortex (BUREŠ and BUREŠOVÁ[3]) by SD.

Methods. Bipolar silver electrodes were implanted in the dorsal hippocampus, RFT and Hy (in area hypothal. lat., only in some cases in zona incerta) as was checked histologically (FIFKOVÁ). Rectangular pulses (450 cy/sec, $t < 0.5$ msec, duration of the salves 60 msec) were given at intervals of at least 2 min, but only when spontaneous synchronization in the hippocampus was not present. A constant level of threshold being established for control, unilateral SD was evoked by local application of filter paper (2 × 2 mm) soaked with 25% KCl on the dural surface. After re-estimating the threshold bilateral SD was elicited.

Results. The cortical SD is accompanied always by a rise of thresholds. In no case does the threshold decrease. The increase of threshold was more pronounced in the tegmentum than in Hy (in the latter not significant, see Table). Between the threshold changes in these two areas

	SD ipsilateral to side of stimulation	SD contralateral to side of stimulation	SD bilateral
Tegmentum			
Elevation of threshold (in % of cases)	82.6	80.0	85.8
Number of experiments	11	5	14
Mean elevation of threshold (M) ± mean error (m) %	124.5 ± 6.0*	117.2 ± 8.5*	168.4 ± 15.4*
Significance against control (100%)	$p = 0.01$	not significant	$p = 0.003$
Hypothalamus			
Elevation of threshold (in % of cases)	57.1	20.0	54.5
Number of experiments	7	5	11
Mean elevation of threshold (M) ± mean error (m) %	108.1 ± 4.9*	108 ± 8.0*	109 ± 4.5*
Significance against control (100%)	not significant	not significant	not significant
Significance between values with * marked	$p = 0.05$	not significant	$p = 0.003$

[1] J. D. GREEN, *The Hippocampus*, in *Handbook of Physiology, Neurophysiology* (Washington 1960), II, p. 1373.
[2] T. WEISS and E. FIFKOVÁ, EEG Clin. Neurophysiol. *12*, 841 (1960).
[3] J. BUREŠ and O. BUREŠOVÁ, EEG Clin. Neurophysiol., Suppl. 13, 359 (1960).



Hippocampogram during stimulation of tegmentum (1a) and hypothalamus (1b). (A) Control (1 = spontaneous synchronisation, 2 = subthreshold stimulation, 3 = threshold stimulation, 4 = suprathreshold stimulation). (B) During ipsilateral SD (1 = subthreshold, 2, 3 = threshold). (C) During bilateral SD (see B). Arrows = onset of stimulation (numbers indicate percentage of threshold voltage).

there was a clear difference. As to the reticular stimulation, we found the bilateral SD more effective than the unilateral one, no difference being found between ipsi- or contralateral SD (typical records see Figure).

Discussion. The results of tegmental stimulation are in accordance with experiments on the influence of SD on other indicators of reticular excitability (RÜDIGER, in press). The cerebral cortex has, therefore, a pronounced influence on reticular functions. In our case, an elimination of tonic facilitatory mechanisms can play a role. This fits well with our results about changes of arousal effectiveness of lateralized peripheral stimuli during unilateral cortical SD (WEISS and BUREŠ[4]). Furthermore, it is improbable that SD acts directly at other levels than the tegmentum, as was proved especially for the hippocampus by WEISS and FIFKOVÁ[2]. As to the low threshold rising effect in the Hy, one might account for the existence of two systems (synchronizing, desynchronizing) influencing the hippocampogram antagonistically (YASUKOCHI[5]).

Zusammenfassung. Vorübergehende ein- oder beidseitige funktionelle Dekortikation mittels «spreading depression» ergibt bei elektrischer Reizung des Tegmentums oder Hypothalamus einen Schwellenanstieg des Weckeffektes. Die Reaktionsunterschiede der Hirnteile werden über die theta-Aktivität im Hippokampus beurteilt.

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Institute of Physiology, Czechoslovak Academy of Sciences, Prague (CSSR), August 16, 1961.

[4] T. WEISS and J. BUREŠ, Physiol. bohemoslov. *8*, 393 (1959).
[5] G. YASUKOCHI, Fol. psychiatr. neurolog. japon. *14*, 260 (1960).
[6] Present address: Berlin N4, Pflugstrasse 22.

Lipids in the Silk-Glands of *Bombyx mori* and their Probable Role in Secretory Metabolism

Lipids in the form of spherical or subspherical bodies are ubiquitous in the various protein-secreting cells. The cytologists have usually described such lipid particulates under various names, such as 'osmiophilic bodies'[1,2], 'Golgi bodies'[1,2], 'neutral red bodies'[3]. Very little is understood about their role in the economy of the protein-secreting cells. These bodies are usually considered as the sites of condensation of various secretory products arising in the cytoplasm[1-3]. This suggestion, pioneered by the elegant work of HIRSCH[4] on the vertebrate pancreas, has been confirmed and extended in a variety of cells secreting globular proteins (mainly enzymes) or even other substances like fats, hormones etc.[1]. In this laboratory, KANWAR[5] has fully confirmed and extended HIRSCH's research in a variety of exocrine cells of a number of vertebrates. However, as far as we are aware, no such data are available on the cells secreting fibrous proteins.

Lepidopteran silk glands, with a unique singleness of purpose, provide an excellent material for studies on protein secretion. These glands are usually divisible into three regions, differing widely in the manifestations of the secretory activity; the wall of the glands is single-celled in thickness with a lumen for storing secretion. Moreover, the synthesis of silk proteins by the cells is a continuous process, starting very slowly in early instars, reaching its peak in the 5th instar and terminating in the cocoon-forming larvae, unlike the other protein-secreting glands mentioned above where the secretion occurs in cycles overlapping each other. Thus the accumulation and subsequent consumption of any substance in the cells of the silk glands during activity can reasonably be associated with the secretory metabolism.

When the gelatin sections of the silk glands in *B. mori* are fixed in formaldehyde calcium, postchromed and coloured subsequently with ethanolic Sudan black B[6], the sudanophil lipids usually appear in the form of a few granules dispersed in the cytoplasm and some irregular bodies aggregated under the outer cell membrane (Figure 1). Almost all these sudanophil lipid bodies react like phospholipids in acid haematein test[7]. The various unmasking procedures after BERENBAUM[8], CLAYTON[9], GUPTA[10], designed to reveal bound lipids, did not yield results of any greater importance.

However, the techniques of SERRA[11] produced results worthy of attention. The sections of the silk glands thus processed revealed numerous homogeneous sudanophil spheres varying in their size and having characteristic distribution in each region (Figure 2 and 3). The lipid nature of these sudanophil spheres is confirmed (1) by the lysochrome effect[11,12], (2) the complete lability of their sudanophilia to the prior extraction in lipid solvents[11] (Figure 4), (3) their strongly osmiophilic nature in WIGGLESWORTH[13] technique of buffered OsO_4/ethyl gallate, and (4) their strong affinity for neutral red used supervitally.

Besides an abundant and uniform distribution in the cytoplasm, the lipid spheres are concentrated at the outer and inner cell borders in the secretory and storage regions of the silk glands from 3rd and 5th instar *B. mori* (Figure 2). In the duct of 3rd instar, the lipid spheres form a prominent ring (Figure 3) at about 1/3 of the cells; in 5th instar this ring of lipid spheres moves inwards to lie contiguous to the intima propria. Besides that, the outer cell border continues to have lipid concentration as in the other two regions. In the larvae removed from two-days old cocoons, however, the cells in the secretory and storage regions show only a thin layer of sudanophil material at the inner and outer borders with almost negative cytoplasm (Figure 5); while the duct cells contain only sparsely distributed lipid spheres in the cytoplasm (Figure 6). Thus a clear depletion (consumption) of the lipid material in the activity of the glands during the 5th instar has taken place.

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