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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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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It has been suggested by JUNQUEIRA[14], and JUNQUEIRA and HIRSCH[15], that the energy requirements of the cells for secretory metabolism come from oxidation-reduction processes through Krebs' cycle rather than glycolysis. Such requirements in the silk glands must be quite high, because the energy is required not only for ion transports and synthesis of protein molecules, but also for the passage of secretory products, through thick intima of the cells, its orientation into fibres and the spinning of silk thread. The duct cells may be involved in the latter process only, as no proteins seem to be secreted by them; this is shown by the absence of any pentose nucleoproteins in their cytoplasm – a substance considered pre-requisite for protein synthesis[16].

It has been further observed that the cells of silk glands are completely negative to periodic acid-Schiff and other[17] tests for carbohydrates. The cytoplasm of these cells is rich in mitochondria having a distribution pattern tallying closely with that of lipids. The cytoplasm during 3rd and 5th instars also contains quite high activity of true lipases[18], and esterases[19] but almost none in the old larvae. The succinic dehydrogenase and cytochrome oxidase activity of the mitochondria also show similar

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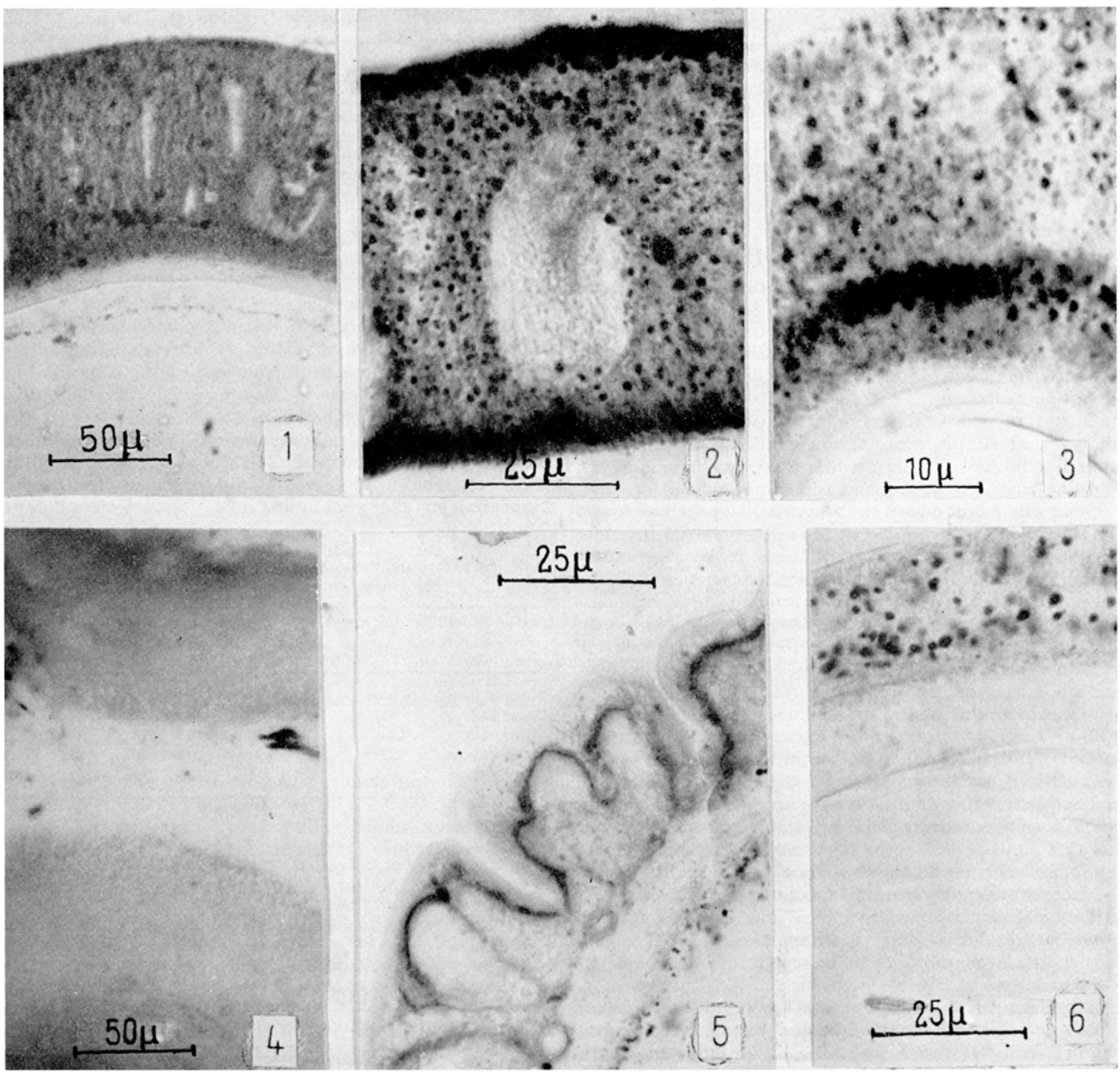

Figures 1–6. Transverse sections of the silk glands of *B. mori*. 1. Formaldehyde calcium fixed postchromed/gelatin section coloured with ethanolic Sudan black B, 5th instar secretory region. 2. Serra (11) fixation demasking coloration mixture, 5th instar secretory region. 3. Serra (11) fixation coloration mixture 3rd instar duct region. 4. Serra (11) extraction mixture, 5th instar secretory region. 5. Serra (11) demasking coloration mixture 2-days old cocoon secretory region. 6. Serra (11) demasking coloration mixture, 2-days old cocoon, duct region.

variation as observed visually with cytochemical tests[20]. Succinic dehydrogenase activity seems to be the highest in 5th instar larva.

It has been shown recently by FUKUDA et al.[21] that 70% of the silk protein in *B. mori* is derived directly from the proteins of mulberry leaves but the remaining 30% is synthesized from the tissue proteins of the larvae. The latter synthesis occurs only after the 7th day of the 5th instar larva.

Thus it can be concluded from the above evidence that up to about the middle of 5th instar there is lipid synthesis and storage in the cells of the silk glands (high lipase activity but low succinic dehydrogenase activity[20]), while during the last phase of the 5th instar and early cocoon-forming larvae there is lipid depletion. It would appear that these lipids are required for the synthesis of 30% of the silk proteins from the larval tissue proteins[21], probably for the production of energy for secretory metabolism as there are no lipids present in the secretion itself.

Lastly it may be pointed out that evidence is accumulating in favour of lipids providing the fuel for certain type of metabolic activity[22,23]. Could it be that the visible consumption of lipids during a secretory cycle in the exocrine cells of vertebrate pancreas (and also other cells) observed by KANWAR and BAKER[24] also meets the same purpose?

Résumé. Dans les glandes séricigènes des mues de la chenille du *Bombyx mori*, les lipides sudanophiles s'accumulent progressivement jusqu'à la 5e mue et s'épuisent complètement pendant la formation du cocon. Une étude systématique des divers systèmes d'enzymes montre que les lipides fournissent par oxydation-réduction l'énergie nécessaire au métabolisme sécrétoire des cellules des glandes pendant les dernières mues.

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Agar Electrophoresis of Soluble Proteins Isolated from Cellular Fractions of Regenerating Guinea-Pig Liver

The changes occurring in regenerating rat liver soluble proteins has been widely studied by means of paper electrophoresis.

GUIDOTTI and CLERICI[1] and GUIDOTTI et al.[2] have shown that simple proteins are practically unmodified after partial hepatectomy, while the periodic acid-Schiff staining fractions were found to be markedly decreased.

Otherwise a decrease of the albumin-like peak was found by DE LAMIRANDE et al.[3,4] in the mitochondrial fraction.

Since the agar gel electrophoresis is very advantageous in obtaining an improved displacement of the soluble cellular proteins[5], we have studied the protein pattern of regenerating liver by means of this technique. In the present paper results are reported on the fractionation of guinea-pig liver soluble proteins, isolated from nuclear, mitochondrial and cytoplasmatic fractions.

Material and Methods. Male guinea-pigs, of about 230–270 g, England strain, were partially hepatectomized (about $^1/_3$ of the total liver), according to HIGGINS and ANDERSON[6], and killed after 24, 48, 72 h and 6, 8 days.

The perfusion of the liver was carried out at 4 C° with the following solution: NaCl 0.094 Mol, phosphate buffer pH 7.4 0.012 Mol, ethylene-diaminetetra-acetic acid 0.011 Mol, glucose 0.046 Mol.

Nuclear and mitochondrial fractions were obtained by differential centrifugations[7,8–10]. The soluble proteins have been isolated after the disruption of nuclear and mitochondrial membranes by means of repeated freezings and thawings.

After dyalisis, centrifugation (for removing proteins which are insoluble in salt-free water) and freezing-dry, the material was dissolved in the buffer solution, at the concentration of about 3–4%.

The technique of agar electrophoresis of GRABAR[11] was essentially followed.

Results. For the nuclear fraction of normal liver 10 components have been observed: 1 in albumin, 1 in α_1-globulin, 2 in α_2-globulin, 4 in β-globulin and 2 in γ-globulin zone. In Table I are reported the percentual amounts of different protein fractions.

After hepatectomy two distinct components in pre-albumin zone appeared (after 24 h, with a maximum after 3 days); moreover an increase of the albumin-like fraction, and a decrease of the components corresponding to β- and γ-globulins, has been observed.

For the mitochondrial preparation, 7 components in the normal liver were observed: 1 in albumin, 1 in α_1-globulin, 2 in α_2-globulin, 3 in β-globulin zone; no component appeared in the γ-globulin zone. The electrophoretic

Tab. I. Percentual amounts of the soluble proteins isolated from regenerating guinea-pig liver nuclear fraction

	Pre-albumin	Albumin	Globulins α_1	α_2	β	γ
Normal liver	0.03	22.0	15.3	33.3	24.0	7.0
After 24 h	2.3	24.1	23.2	28.1	20.0	2.3
After 72 h	10.4	30.5	10.5	30.6	17.0	1.0
After 6 days	8.6	27.8	10.9	30.1	21.1	1.5
After 8 days	0.4	22.1	13.7	31.5	31.5	1.0

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