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

	Pre-albumin	Albumin	Globulins α_1	α_2	β	γ
Normal liver	0.03	28.0	28.0	35.3	8.7	—
After 24 h	1.2	26.7	18.5	38.9	14.0	0.7
After 72 h	4.9	31.3	11.7	31.8	19.8	1.2
After 6 days	1.0	21.9	13.4	35.3	21.9	6.5
After 8 days	—	16.6	14.7	36.0	32.7	—

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

	Pre-albumin	Albumin	Globulins α_1	α_2	β	γ
Normal liver	0.9	22.2	17.5	33.4	18.4	7.6
After 24 h	3.3	28.2	10.5	25.3	25.1	7.6
After 72 h	2.2	25.7	9.6	29.2	26.7	6.6
After 6 days	2.8	27.2	9.6	29.0	25.6	5.8
After 8 days	1.2	19.8	8.6	22.8	40.0	7.6

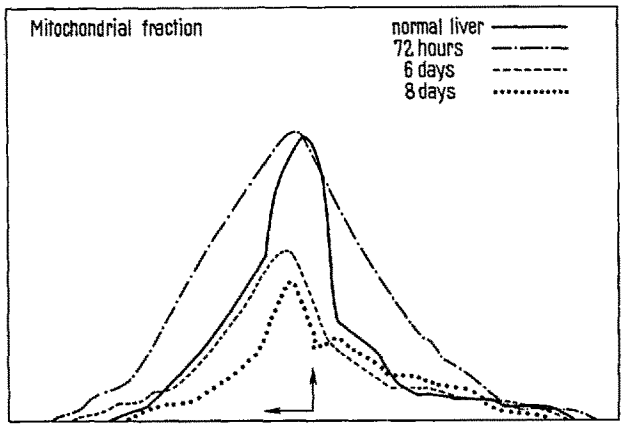

Fig. 2. Electrophoretic patterns of soluble proteins isolated from the mitochondrial fraction of regenerating guinea-pig liver. Preparation according to HOGEBOOM and SCHNEIDER. Buffer: sodium barbiturate 0.065 Mol; hydrochloric acid 0.017 Mol (pH 8.2, ionic strength μ = 0.05). V/cm 6.4; mA/cm 2.5 for 7 h. Staining with Amidoschwarz 10B.

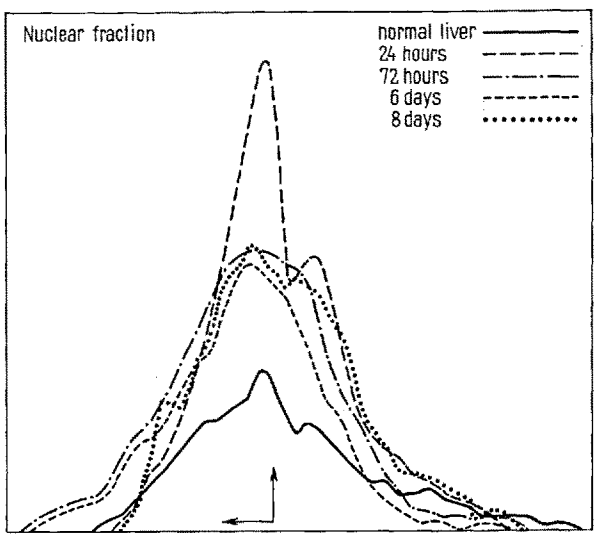

Fig. 1. Electrophoretic patterns of soluble proteins isolated from the nuclear fraction of regenerating guinea-pig liver. Preparation according to HOGEBOOM and SCHNEIDER. Buffer: sodium barbiturate 0.065 Mol; hydrochloric acid 0.017 Mol (pH 8.2, ionic strength μ = 0.05). V/cm 6.4; mA/cm 2.5 for 7 h. Staining with Amidoschwarz 10B.

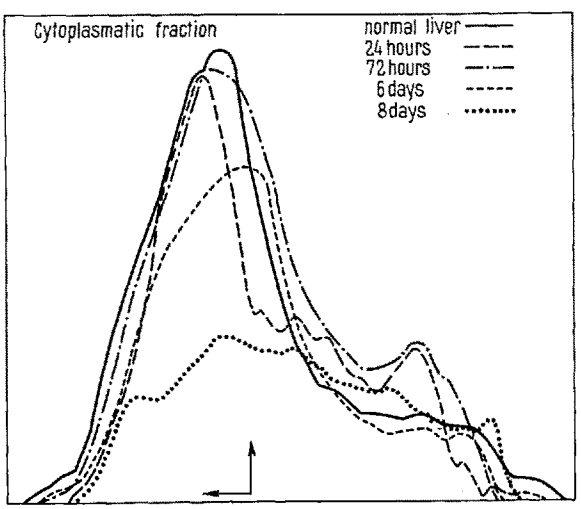

Fig. 3. Electrophoretic patterns of soluble proteins isolated from the cytoplasmatic fraction of regenerating guinea-pig liver. Preparation according to HOGEBOOM and SCHNEIDER. Buffer: sodium barbiturate 0.065 Mol; hydrochloric acid 0.017 Mol (pH 8.2; ionic strength μ = 0.05). V/cm 6.4; mA/cm 2.5 for 7 h. Staining with Amidoschwarz 10B.

patterns of the hepatectomized guinea-pigs showed a marked increase of the less dispersed fractions, after 6 and 8 days. The appearance of a small prealbumin component was also noted for the mitochondrial preparation, after 72 h (Table II).

The combined supernatants obtained in the isolation of mitochondria, that we have called 'cytoplasmatic fraction', contain microsomes with the soluble material of the homogenates; since this preparation was also submitted to repeated freezings and thawings, the microsomial proteins were largely extracted.

This preparation showed, in the normal liver, 10–12 well defined components, with the percentual amounts reported in Table I.

In the regenerating liver all the components with a mobility corresponding to prealbumin, albumin and β-globulin peaks were found to be increased, while a decrease of the α_1-and α_2-globulin fractions was observed. The most striking result was represented by the appearance of a new component, which migrates between β- and γ-globulin fractions, with a great evidence after 24 and 72 h.

The finding of the increase of prealbumin and albumin components in the nuclear proteins preparation, occurring 24 and 72 h after hepatectomy, may suggest a relation with the increase of DNA nuclear content, observed by TSUBOI et al.[12,13].

Otherwise, the marked increase of the less dispersed protein fractions for mitochondrial and cytoplasmatic materials, and the appearance of a new component between β- and γ-zone, could probably be explained by the higher synthesis of the structured cytoplasm proteins,

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according to the augmented number and volume of the regenerating liver mitochondria, as observed by TSUBOI et al.[12] and COSTA et al.[14].

Riassunto. Gli autori hanno studiato, mediante elettroforesi su agar, il comportamento delle proteine solubili delle frazioni cellulari isolate dal fegato di cavia in corso di rigenerazione.

A carico della frazione nucleare e di quella citoplasmatica è stato osservato un aumento percentuale dei componenti proteici più veloci, mentre nella frazione mitocondriale sono aumentati i componenti meno dispersi. Nella frazione citoplasmatica si è notata la comparsa di un nuovo componente, con velocità intermedia tra le β- et le γ-globuline.

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Senkung der Körpertemperatur bei Mäusen durch Librium

Sämtliche bisher bekannten psychotropen Drogen senken die Körpertemperatur von Laboratoriumstieren, zum Beispiel Reserpin und Serotonin[1], zum Teil auch LSD-25[1], ferner Chlorpromazin[1,2] und andere Phenothiazine[3]. Eine neuartige Droge, die sich in ihren Wirkungen von anderen Tranquillizern dadurch unterscheidet, dass sie die Aggressivität herabsetzt, ohne die allgemeine motorische Aktivität zu beeinträchtigen[4], stellt «Librium» Roche (7-Chlor-2-methylamino-5-phenyl-3-H-1,4-benzodiazepin-4-oxyd-hydrochlorid) dar. Dieser Unterschied gegenüber der pharmakologischen und therapeutischen Wirkung anderer Tranquillizer erlaubt die Frage, ob auch unter Librium eine Senkung der Körpertemperatur eintritt.

Wir verwendeten männliche Mäuse von 20 g Durchschnittsgewicht. Die Körpertemperatur wurde mit dem «Ellab»-Gerät (Elektrolaboratoriet Kopenhagen) gemessen; die Rektalfühler wurden etwa 2 cm tief eingeführt und während der Messperiode (2 h) durch Fixation am Schwanz belassen. Die Messung erfolgte in Intervallen von 5 min. Umgebungstemperatur: 22,5°C. Librium wurde intraperitoneal in verschiedenen Dosen verabreicht.

Einige Ergebnisse zeigt Figur 1; aus ihr wird ersichtlich, dass nach höheren Dosen ein (signifikanter) Abfall der Körpertemperatur um etwa 4°C eintritt. Nach niederen Dosen bleibt der Abfall der Körpertemperatur zum Teil

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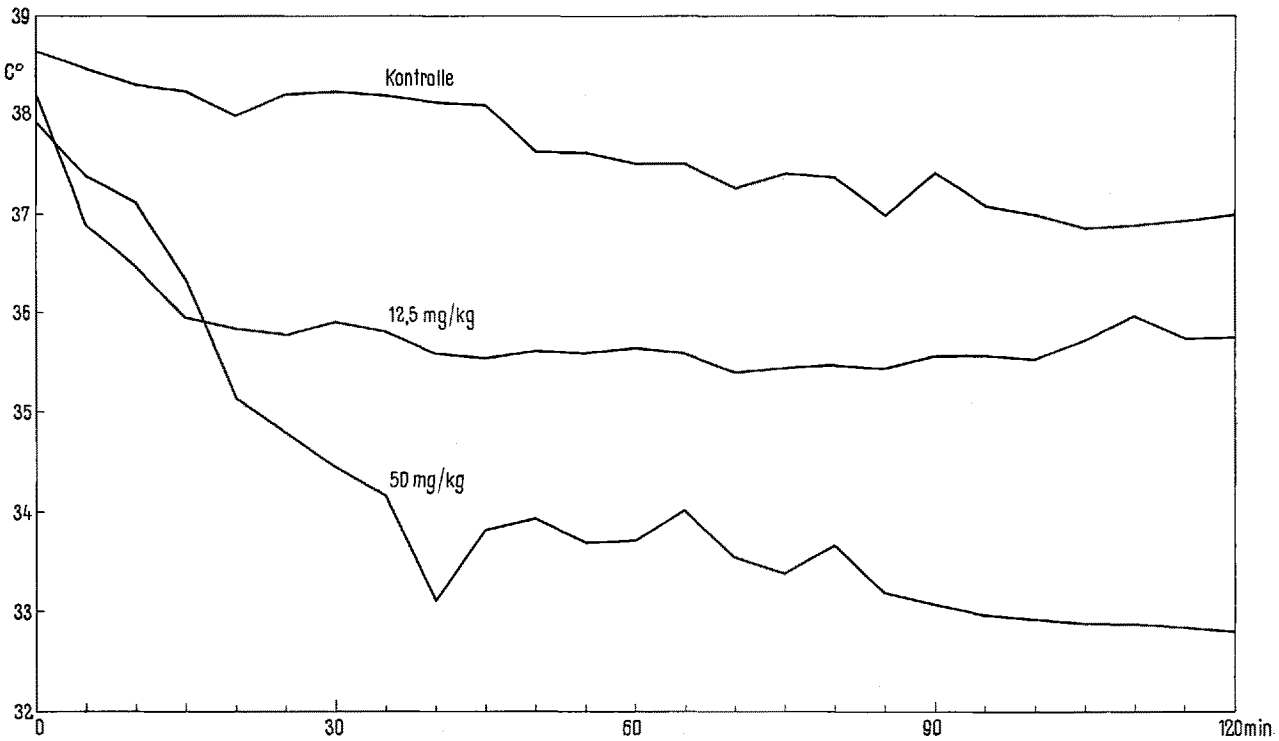

Fig. 1. Verlauf der Körpertemperatur von Mäusen unter Einfluss von Librium angegebener Dosierung (Durchschnittswerte von jeweils 6 Einzelversuchen) im Vergleich zu Kontrolltieren (Durchschnitt von 5 Einzelversuchen). Ordinate: Körpertemperatur in °C, Abszisse: Zeit in min.