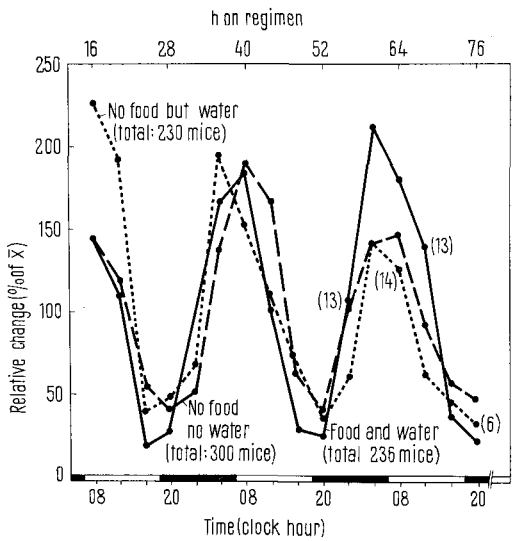
to all animals until 16 h prior to the first sampling, when food was removed from the cages in one room while food and water were both removed from the cages in another room. The animals in the third room were checked (to provide a similar disturbance), but had food and water available ad libitum throughout the whole sampling span.

Subgroups of approximately 15 mice from each of the three groups were sacrificed at 4 h intervals. The liver was immediately removed, frozen, and the glycogen concentration determined by the method of KEMP and KITS VAN HEIJNINGEN[11].

In the animals with food and water ad libitum, the liver glycogen concentration varied predictably as a function of time, from mean crest values of about 8.0 g glycogen/100 g tissue wet weight to trough values as low as 0.36 g. The starving and thirsting as well as the starving animals showed on inspection of time plots similar circadian rhythms that were roughly comparable also with the rhythm in the fully-fed and fully-watered mice, as can be seen from the relative values shown in the Figure – although the absolute liver glycogen levels in starving groups of animals ranged from a mean value of 0.356 g glycogen/100 g tissue wet weight at the circadian crest time to 0.049 g at the time of trough.



Chossat phenomenon in hepatic glycogen of the mouse (see text). Liver glycogen concentration in C female mice, 4.5 months of age (15 mice per point, unless otherwise noted in parantheses).

At the extremely low levels of liver glycogen in the starved and dehydrated mouse, the circadian rhythm persists and its amplitude during the first day of dehydration and/or starvation does not compare unfavorably with that in controls. Apart from the practical interest in these results related to a biosatellite survey of biochemical and other rhythms, the Figure demonstrates for yet another function what might be called, in honor of a pioneer in this field, a CHOSSAT[12] phenomenon[13].

Zusammenfassung. Eine Circadianrhythmik des Mäuseleberglycogens lässt sich durch Standardisierung anderer biologischer Frequenzen und «Lärm» isolieren. Dies gelingt auch nach Entzug von Futter oder Futter und Wasser. Persistente Rhythmen mit grosser Amplitude kennzeichnen eine Reihe physiologischer Funktionen nach Futter- und Flüssigkeitsentzug und können als Chossatphänomene bezeichnet werden.

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Hexamethylentetramine-Silver Reaction for Lysosomes

In the course of a study (DE MARTINO et al.[1]) about the feasibility of the application of the hexamethylentetramine-silver technique in order to stain various cytoplasmic structures, silver-stained granules were observed in the parenchymal cells of the liver and in those of the intestinal epithelium. Similar granules were also stained in the proximal convoluted tubules of the kidney. This study was performed to assay the specificity of the reaction and to define the identity of the stained granules.

Kidney, liver and small intestine of adult newts (*Triturus cristatus* Laur.) were used in this study. Four different fixatives were used: Sanfelice, Bouin, Carnoy and acetic sublimate. 5 μ paraffin sections, after oxidation with 10% H_5IO_6 (15–20 min), were exposed to silver hexamethylentetramine (5–12 h). The silver solution was prepared according to the methods of JONES[2] and MARINOZZI[3] for the basal membranes, as modified by DE MARTINO et al.[1]. Other sections from the same block were oxidized with 10% $NaIO_4$ before silver impregnation. Frozen sections, 8–10 μ thick, from formol-calcium fixed tissues, were incubated for acid phosphatase activity to demonstrate the presence of lysosomes by the GOMORI[4] procedure.

In the liver, many silver-stained granules were localized around the bile canaliculi. Similar granules were also stained in the proximal convoluted tubules of the kidney and in the supranuclear region in the intestinal epithelial cells. These granules matched in size and distribution the lyosomes demonstrated by the Gomori technique. However, in the intestinal cells, these granules were revealed by the silver reaction only when the material had been fixed in Sanfelice or in Bouin.

The results obtained in our study confirm and extend the observations of other authors. LILLIE[5] described

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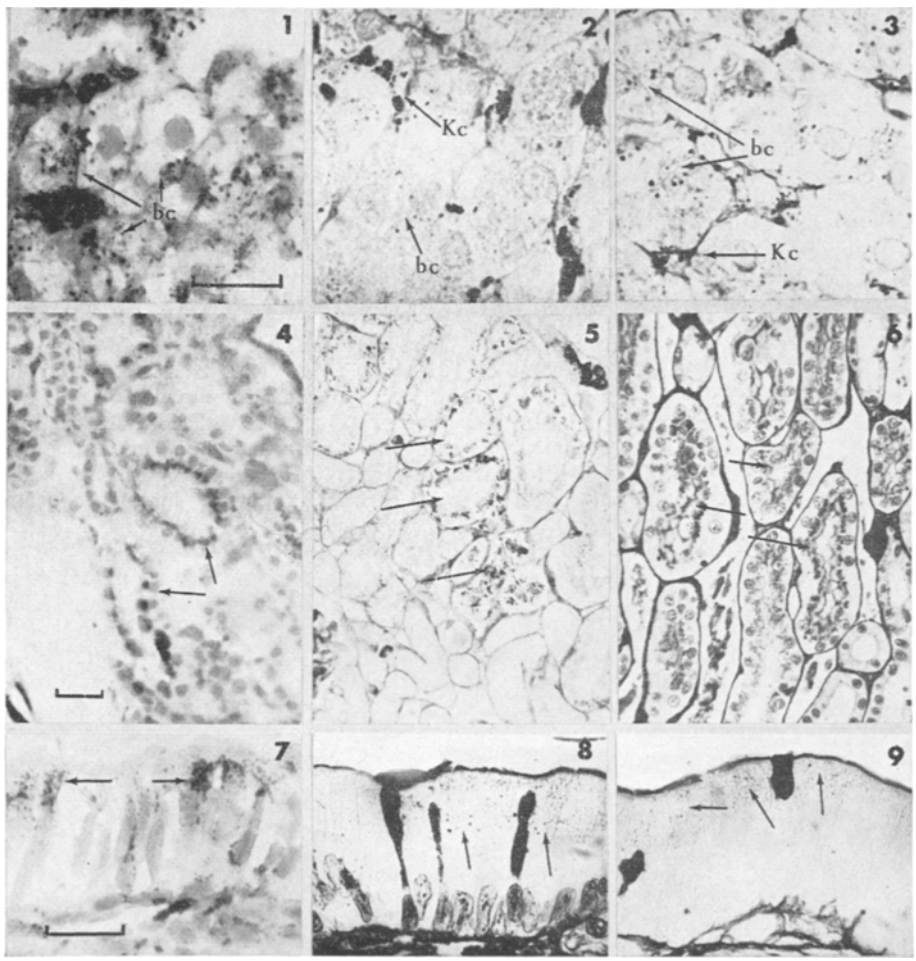

Fig. 1. Liver. Gomori's method for acid phosphatase. Numerous lysosomes can be seen around the bile canaliculus (bc).
Fig. 2. Liver. Fixation in acetic sublimate. $NaIO_4$ oxidation. Silverhexamethylentetramine staining. Silver-positive granules are located around a bile canaliculus (bc). A Kupffer cell is also visible (Kc).
Fig. 3. Liver. Fixation in Bouin. $NaIO_4$ oxidation. Silver-hexamethylentetramine staining. The distribution of the granules is the same as in the preceding Figures.
Fig. 4. Kidney. Gomori's method for acid phosphatase. The lysosomes are visible in the cells of proximal tubules (arrows).
Fig. 5. Kidney. Fixation in acetic sublimate. $NaIO_4$ oxidation. Silver-hexamethylentetramine staining. Numerous silver-positive granules are present in the tubular cells.
Fig. 6. Kidney. Fixation in Sanfelice solution. $NaIO_4$ oxidation. Silver-hexamethylentetramine staining. The distribution of the granules is the same as in the preceding Figures.
Fig. 7. Intestine. Gomori's method for acid phosphatase. The intestinal cells contain numerous lysosomes (arrows).
Fig. 8. Intestine. Fixation in Sanfelice solution. $NaIO_4$ oxidation. Silver-hexamethylentetramine staining. Silver-positive granules are present in the intestinal cells.
Fig. 9. Intestine. Fixation in Bouin solution. $NaIO_4$ oxidation. Silver-hexamethylentetramine staining. The distribution of the granules is the same as in the preceding Figures.

silver-positive granules in megakaryocites and leucocytes and COHN, HIRSCH, and WIENER[6] demonstrated that these granules were lysosomes. SANDBANK and BECKER[7] stained the lysosomes in cells of the central nervous system and kidney tubules by means of Hortega's ammoniated silver carbonate.

Our present research demonstrates that, in the liver, in the kidney and in the intestine, the silver hexamethylentetramine reaction stains cytoplasmic granules which, for their form, size and distribution, correspond to similar bodies demonstrated by the Gomori technique for acid phosphatase activity and are widely recognized as lysosomes (NOVIKOFF, BEAUFAY, and DE DUVE[8]; ESSNER and NOVIKOFF[9]; MAUNSBACH[10]; MILLER and PALADE[11]; HSU and TAPPELL[12]). In 1962 KOENIG[13] and KOENIG and BARRON[14] observed that the periodic acid-Schiff method stained lysosomes and postulated that lysosomes contained a glycoprotein.

It can probably be assumed that the silver-hexamethylentetramine reacts with aldehyde groups released from the glycoproteins of the lysosome matrix during the oxidation. The basis for this staining would therefore appear to be similar to that of the periodic acid-Schiff method, but the discreteness and the intensity of the staining are much higher. Thus, the silver-hexamethylentetramine procedure appears to be a useful, non-enzymatic method for the demonstration of lysosomes in animal tissues, fixed and embedded according to current histological techniques[15].

Riassunto. È stata proposta una tecnica non enzimatica per mettere in evidenza i lisosomi nei tessuti animali; essa è basata sulla affinità che l'argento-esametilentetrammina, dopo ossidazione ($NaIO_4$ o H_5IO_6), ha per la matrice lisosomiale, riconosciuta, da precedenti autori, come lipoglicoproteica.

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