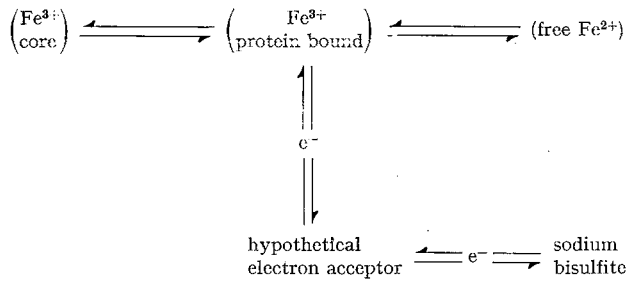
Whereas the shape of the curves was easily reproduceable, the absolute values of ΔE depended on the quality of the sodium bisulfite. The experiment shows the decoloration of β-ferritin to proceed more rapidly than that of α-ferritin.

Discussion. No difference in iron release between α- and β-ferritin would be expected if the sodium bisulfite reacted directly with the (FeOOH) core. It could be postulated, therefore, that sodium bisulfite reacts at first with a primary electron acceptor, possibly a specific electrophilic group in the protein moicty of ferritin. The overall reaction of ferritin with sodium bisulfite could then be written as



It is conceivable that the curve in the Figure is due to differences in the postulated primary electron acceptor.

One could argue that more iron release sites were available in the β-fraction due to a higher concentration of β-ferritin. This would indeed be the case if the iron content per molecule were lower in the β-ferritin than in the α-ferritin. This could be ruled out, however, by the demonstration of identical iron/protein ratios in the two molecules.

The work of JONES[3] suggests that delivery of Fe^{3+} by the (FeOOH) micelle follows first order kinetics. Hence in a hypothesis without primary electron acceptor a lower iron content of α-ferritin could not be made responsible for the slower iron release.

The observation of difference in iron release by α- and β-ferritin fits KOPP's[4] suggestion that the tendency of β-ferritin to incorporate iron may be more pronounced than that of α-ferritin. However, the biological significance of delayed iron uptake and release by α-ferritin is not understood.

Zusammenfassung. Es wurde beobachtet, dass α-Ferritin unter der Einwirkung von Natriumhydrosulfit sein Eisen langsamer abgibt als β-Ferritin. Diese Beobachtung lässt vermuten, dass das dreiwertige Eisen zunächst eine Bindung mit dem Eiweiss eingeht und erst dann durch einen primären Elektronenakzeptor reduziert wird. Ein Unterschied im primären Elektronenakzeptor zwischen α- und β-Ferritin könnte die verschieden rasche Eisenabgabe erklären.

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Nucleic Acid, Amino Acid, and Carbohydrate Metabolism of Nurse Cell Nucleoli in *Musca domestica*

The 15 highly polyploidisized nurse cell nuclei (NCN) in the polytroph meroistic egg follicles of *Musca domestica* (Figure 1a) develop a great number of nucleoli in the course of oogenesis. Probably the formation of these multiple nucleoli follows on principle the scheme described for the nucleolar apparatus in *Calliphora erythrocephala*[1]. The vital aspect of the NCN-nucleoli of *Musca* is demonstrated in Figure 1b by a Nomarski interference phase contrast micrograph. The NCN-nucleoli attain a maximal size on oogenetic stage 3b[2]. Their DNA-content is much lower than that of the remaining nuclear space. So ^{3}H-thymidine incorporation during s-phase of endomitotic polyploidization exhibit a labelling pattern, in which nucleoli stand out by a very low grain density[3]. Thus they are producing holes in the otherwise homogeneous high label attributed to the chromosomes (Figure 1c).

In this paper some data concerning the RNA-, protein-, and carbohydrate metabolisms of the trophocyte nucleoli will be presented.

Materials and methods. The flies were reared under standard conditions[4] at 21 °C. Females at stage 3 of oocyte development[2] were injected with 5 µl of an aqueous tracer solution containing 5 µC of ^{3}H-uridine (spec. act. 5 C/m*M*), ^{3}H-D-glucose (spec. act. 1.22 C/m*M*), or a mixture of ^{3}H-L-amino acids (valine, tyrosine, leucine, lysine, histidine in equal parts of 1 mC/ml, specific activity between 500 and 1000 mc/m*M*). After 15 or 30 min of incubation the ovaries were dissected, fixed in modified Carnoy's fluid or Gendre's fluid[5], embedded in paraplast, sectioned 10 µm thick, and prepared for autoradiography with Ilford liquid emulsion K.2 or Kodak stripping film AR10.

Results. RNA autoradiography. After 30 min of ^{3}H-uridine incorporation the NCN-nucleoli stand out from the remaining nuclear area by a stronger labelling in the autoradiographs (Figure 2a)[2, 6, 7]. After pretreatment with RNase no further labelling of nucleoli or other cellular components can be detected. By 30 min of ^{3}H-uridine incubation autoradiography demonstrates the precursor to incorporate exclusively into macromolecular RNA.

Protein autoradiography. Also by application of ^{3}H-amino acids (15–30 min), the multiple nucleoli can be identified as sites of the highest labelling in the nurse cells (Figure 2b). After 30 min the grain density of the nucleoli areas even exceeds that of the follicle epithelium, which has been shown to be the cell type with the highest protein turnover[2]. A pretreatment of the sections with RNase (Figure 2c) or diastase has no influence on the pattern and intensity of labelling compared with controls. Therefore it must be concluded that during 30 min of incubation autoradiography comprehends use of amino acids only for protein synthesis.

^{3}H-glucose autoradiography. The same pattern, with comparably high labelling intensity of the nucleoli as

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[8] Wir danken Herrn Prof. Dr. K. BIER für Überlassung der Figur 2a, Herrn C. SCHOLZ für die Figur 1c sowie Frau H. THIESIES für technische Assistenz.

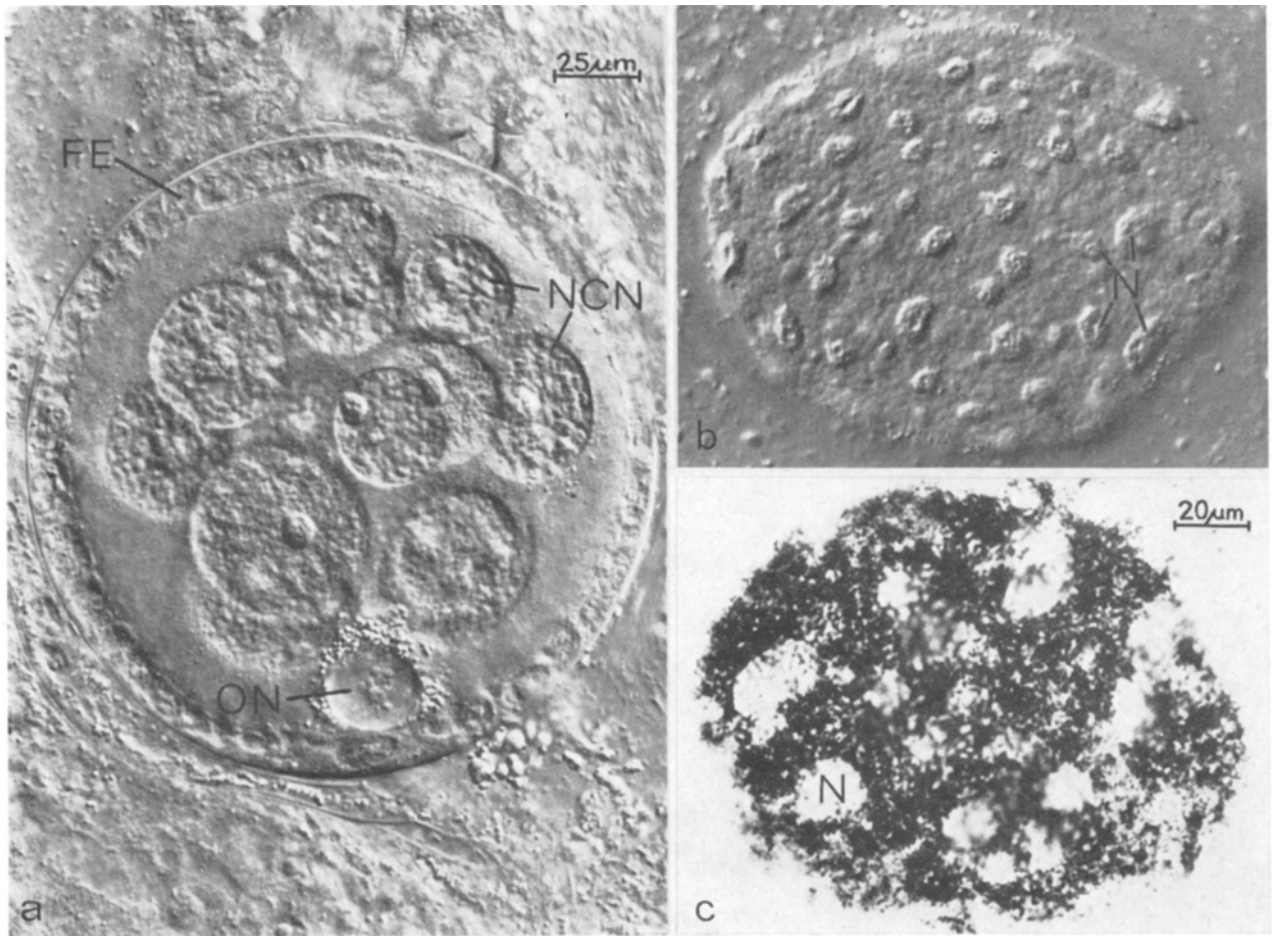

Fig. 1. (a) Egg follicle at stage 3 of development, consisting of the oocyte (ON = oocyte nucleus) and the nurse chamber with 15 trophocytes (14 giant nurse cell nuclei (NCN) are visible), surrounded by a monolayered follicular epithelium (FE). (b) A single nurse cell nucleus of stage 3b containing a great number of nucleoli (N). a and b: living squasch preparations, Nomarski interference phase contrast. (c) Autoradiograph of a nurse cell nucleus, 12 h ^{3}H-Thymidine[8]. The areas of the nucleoli (N) are nearly unlabelled, the rest of the volume of the highly polyploid nucleus is filled with replicating chromosomes.

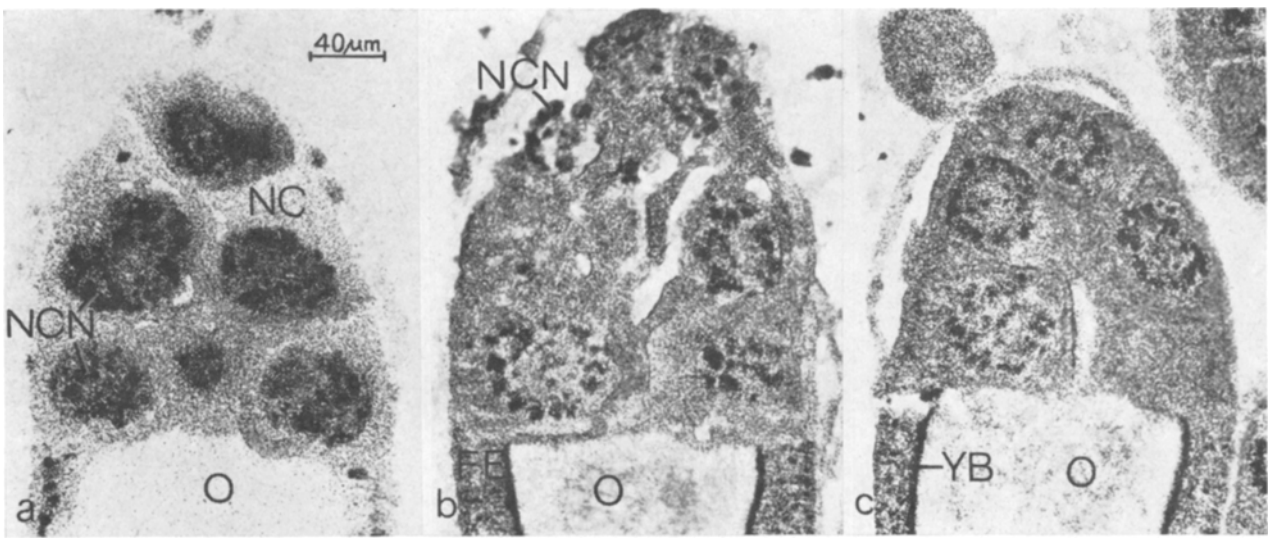

Fig. 2. Nurse chamber stage 3 autoradiographs, 30 min incubation with ^{3}H-uridine (a) or ^{3}H-amino acids (b and c). (a) The highest grain density can be found over the nurse cell nucleoli (NCN). The label of the nurse cell cytoplasm (NC) is due to RNA transport mechanism and has been shown to be RNase-sensitive. In the ^{3}H-amino acids incorporation pattern also the nurse cell nucleoli (NCN) are most prominently labelled (b) but in contrast to the RNA experiment (a) this labelling is not at all affected by RNase pretreatment (c). Between the follicular epithelium (FE) and the oocyte (O) the protein yolk border (YB) is seen.

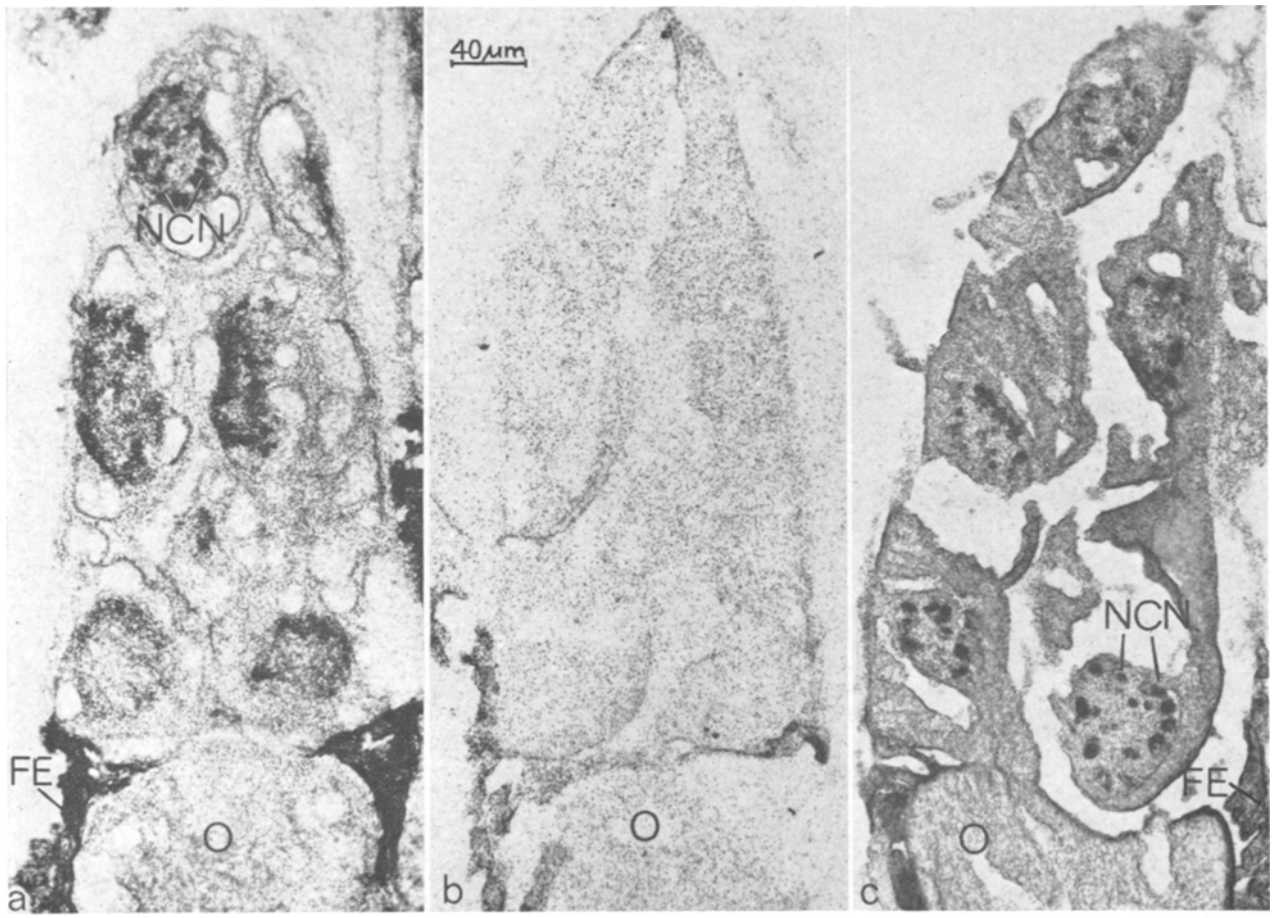

Fig. 3. Autoradiographs of a stage 3b nurse chamber, 30 min ^{3}H-glucose, fixation by Gendre's fluid, which gives the best results in carbohydrate preservation but causes the tissue to crumble. (a) Untreated section showing a strong, nearly elective labelling of the nurse cell nucleoli (NCN) beside which only the follicular epithelium (FE) appears to be considerably radioactive. (b) The RNase-sensitivity of all the nucleolar label demonstrates the utilization of ^{3}H-glucose for RNA synthesis. (c) Diastase digestion does not alter the labelling pattern of the nurse cell nucleoli (NCN) showing that it is not caused by newly synthetized polysaccharides. The oocyte (O) always remains unlabelled, the radioactivity of the follicular epithelium (FE) is only weakly diminished by enzyme treatments.

described above for the incorporation of ^{3}H-uridine and ^{3}H-amino acids, can be obtained after 15 min and 30 min of ^{3}H-glucose application (Figure 3a). Polysaccharids are not detectable in the NCN-nucleoli by the PAS-reaction, even if fixation and mounting techniques of the preparations guarantee an optimal preservation of macromolecular carbohydrates in the slices[5]. A diastase pretreatment does not decrease the grain density over the nucleoli areas (Figure 3c) whereas RNase removes nearly all macromolecules containing the probably metabolized ^{3}H-glucose (Figure 3b). The insignificant residual labelling might be attributed to metabolized ^{3}H-glucose incorporated into proteins.

Discussion. Besides the high RNA metabolism[6], the multiple nucleoli of *Musca* NCN have been proved to be sites of conciderably rapid ^{3}H-amino acid and ^{3}H-glucose incorporation. The strong labelling of the nucleoli after short incubation of ^{3}H-amino acids perhaps really represents a nuclear protein synthesis. Otherwise a relatively rapid transport of newly synthetized proteins from nurse cell cytoplasm into nurse cell nuclei must occur. The use of injected glucose as a precursor for the synthesis of macromolecular nucleolar RNA has been demonstrated by the RNase sensitivity of the autoradiographic pattern. The labelling after ^{3}H-uridine incorporation differs insofar from that after ^{3}H-glucose injection as in the latter case the non-nucleolar areas exhibit a lower grain density already after 15 min of incubation. It seems as if metabolized glucose predominately incorporates into nucleolar RNA during a short incubation. This metabolization of glucose probably into ribose via pentose-phosphate-cyclus is perhaps structurally localized in the nurse cell nucleoli.

The presented data are preliminary results of current investigations.

Zusammenfassung. Die multiplen Nukleolen in den polyploiden Nährzellkernen des Ovars von *Musca domestica* erweisen sich in Kurzzeitexperimenten als Orte des höchsten RNS-, Protein- und Glukoseumsatzes. Die relativ stärkste autoradiographische Markierung der Nukleolen findet sich nach 15–30 min ^{3}H-Glukose-Inkubation. Dieser Befund wird als Hinweis für eine möglicherweise bevorzugt im Nukleolenbereich stattfindende Umwandlung von Glukose in Ribose gewertet, die bei der Nukleotidsynthese für die rRNS-Produktion verwendet wird.

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