Autoradiographic studies on RNA synthesis and transport in the Salivary gland of *Lygaeus* sp. (Hemiptera-Lygaeidae)

D. Kumar[1]

Department of Zoology, Banaras Hindu University, Varanasi 221005 (India), 3 March 1980

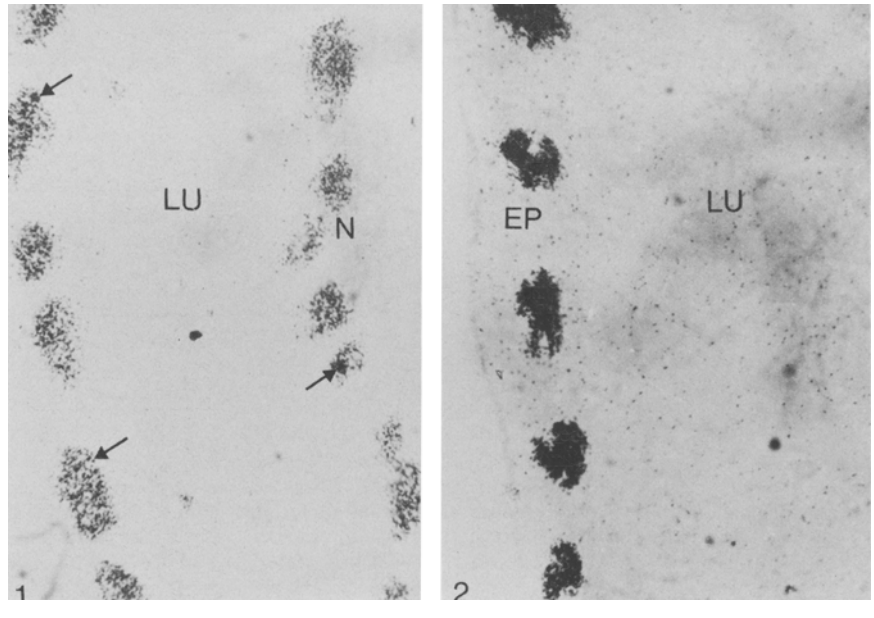
Summary. Autoradiographic studies using ^{3}H-uridine in the salivary gland of adult *Lygaeus* sp. were carried out. The gland cell nuclei, particularly the multiple nucleoli, are the sites of incorporation of the label exhibiting RNA synthesis. The labelled molecules (RNA) are transported to the cell cytoplasm and then into the gland lumen in which no turnover of the radioactivity is observed.

The salivary glands of Hemiptera produce a variety of enzymes used both in piercing and sucking as well as for digesting food material[2]. Most of the enzymes are synthesized by the glandular epithelial cells, although recent studies have shown that haemolymph proteins or their precursors may also be sequestered by the salivary glands[3]. Synthetically active cells are known to produce large amounts of different species of RNA that are involved in the synthesis of proteins (enzymes)[4,5]. RNA metabolism in the salivary glands of insects, particularly in the bugs, is little understood, although it has been investigated repeatedly in polytrophic insect ovaries[6-11]. Our histochemical studies of the salivary glands of *Lygaeus* showed the presence of considerable amounts of RNA in the gland cell cytoplasm and in the lumen[12], but the origin and fate of this RNA was not clear. The present autoradiographic study is an attempt to elucidate this problem.

Materials and methods. Salivary glands of adult laboratory reared *Lygaeus* sp. were used in this study. To investigate the RNA metabolism, the insects were injected with ^{3}H-uridine (sp. act. 2.8 Ci/mmole; dosage 5 µCi/0.05 ml) and incubated for 15 min, 30 min, 1 h, 2 h, 4 h and 6 h. At the end of these incubation times, the salivary glands were dissected out and fixed in Carnoy's fluid. Paraffin sections were processed for autoradiography, using Kodak AR-10 stripping film. Exposure times varied from 24 weeks. Appropriate RNAse controls for the autoradiographs were kept.

Observations. The salivary glands of the insect consist of a pair of principal glands which are lobular structures situated on either side of the oesophagus. Each principal gland is associated with a long, tubular, coiled accessory gland. Histologically, the principal salivary gland is made up of the following structures from outside inward: 1. a 2-layered, inconspicuous peritoneal sheath consisting of squamous cells; 2. a basement membrane on which rest 3. the glandular (single layer) epithelial cells of columnar or cubical shape, with large, highly branched polyploid nuclei, and 4. a spacious lumen containing large amounts of the salivary secretions[12]. The present observations deal with the RNA metabolism in the principal salivary gland.

15-min incubation shows a clearly restricted labelling of the gland cell nuclei and the density of the reduced silver grains is clearly high in the larger nucleoli (figure 1, arrows). The cytoplasmic areas of the cells and the lumen are not labelled. With a 30-min incubation period, the nuclei become more strongly labelled and the radioactivity now begins to appear in the cytoplasm as well as in the lumen (figure 2). With 1-h incubation (figure 3) the incorporation patterns of the label do not show anything special except a steady increase in the level of labelling in the gland cell nuclei, cytoplasm and the lumen contents. It is only with 4-h incubation that one can clearly demonstrate a distinct movement of the radioactivity from the cell nucleus into the cytoplasm (figure 4). One can also clearly see a higher radioactivity of the cytoplasmic regions close to the nuclear



Figs 1-5. Autoradiographs of the salivary gland incubated with ^{3}H-uridine for different periods showing the activity patterns of the nuclei (N), glandular epithelium (EP), and lumen (LU). Magnification of all the figures is ×430.

Fig. 1. 15-min incubation. Incorporation of the label in the gland cell nuclei. Arrows show the nucleoli. Cytoplasm and lumen are completely free of activity.

Fig. 2. 30-min incubation. Nuclei become more radioactive and the movement of the label is seen in the cytoplasm and up to a slight extent in the lumen.

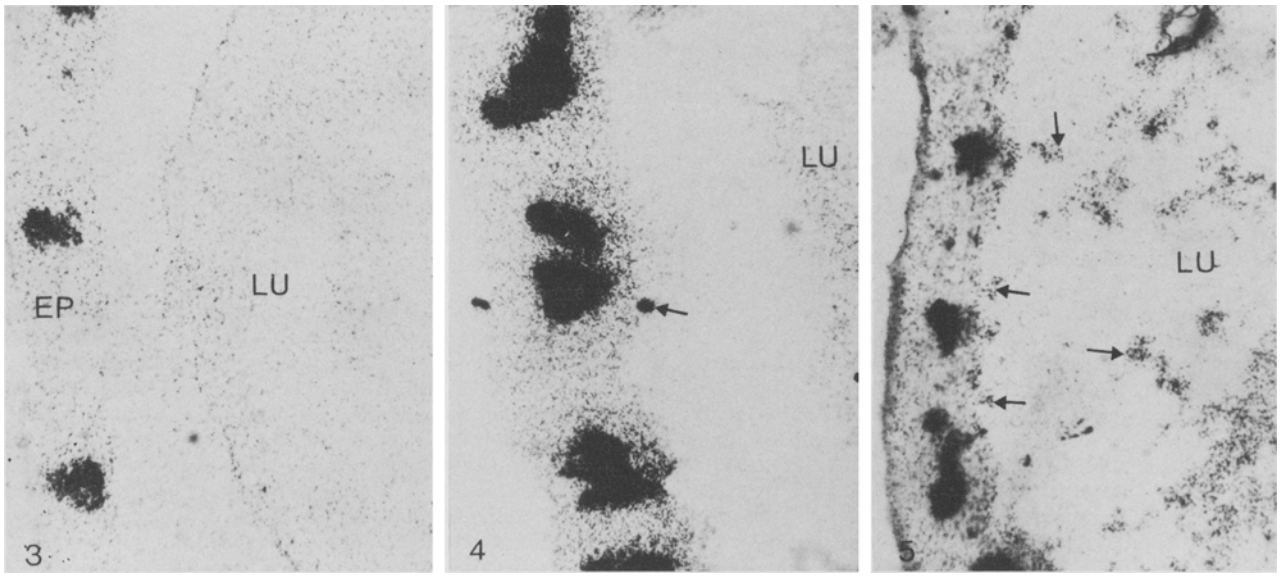


Fig. 3. 1-h incubation. Note the general intensification of the radioactivity in the nuclei, cytoplasm and lumen.

Fig. 4. 4-h incubation. Gland cell nuclei are heavily labelled. The cytoplasm in the apical cells is more radioactive than in the basal ones. A radioactive vesicle (→) is also seen coming into the lumen.

Fig. 5. 6-h incubation. Radioactive molecules are seen coming into the lumen from the apical border (→) and the lumen is heavily labelled.

membrane, compared with the outlying areas of the cytoplasm, so much so that a falling gradient of activity from the nuclear periphery is clearly noticeable. This points to the movement of the radioactivity from the nucleus to the cytoplasm. Nevertheless, the apical cytoplasmic region is more radioactive than the basal one. When the period of incubation is extended up to 6 h, the amount of labelling of the nuclei is reduced and the labelled molecules are released into the lumen (figure 5). Here one can see the clearpinching-off of the radioactive material (figure 5 arrows) at the brush border into the lumen. This phenomenon could be observed with 1-h incubation, but with difficulty, whereas the radioautograph with 4-h incubation (figure 4) also shows a clear radioactive vesicle just being pinched-off from the apical border into the lumen. All these observations show that the gland cell nuclei are involved in an active synthesis of RNA that is discharged into the cytoplasm from where at least some of it is exported into the lumen, and forms a constituent part of the salivary secretion.

The above statement is further supported by the enzymatic studies. When the slides, incubated with the label for various incubation times, were treated with RNAse, it was found that no radioactivity remained either in the gland cell (including nuclei and cytoplasm) or in the lumen, suggesting the removal of RNA molecules from these regions.

Discussion: Since no mitoses occur in the adult salivary gland cells, the nuclei become highly branched and polyploid[12]. The above observations show that the main sites of synthesis of RNA are the multiple nucleoli (cf. figure 1) which is an established fact[13,14]. As the incubation time increases, the RNA synthesis intensifies resulting in the gland cell nuclei becoming completely dark except in their peripheral regions, where reduced silver granules could be observed (cf. figure 2). A further increase in the incubation period produces more RNA, which is transported to the cell cytoplasm from where it is seen flowing into the lumen; no turnover of RNA is observed here. The clear radioactive vesicles at the brush border suggest its RNP (ribonucleoprotein) nature; this is also revealed by our histochemical analyses[12]. The nucleolar origin, and further, its persistence in the lumen, suggests its stable nature, i.e. rRNA; this was the conclusion drawn by Bier[6] for the ovary of *Musca*. Therefore, it is presumed that this RNA which is transported into the gland lumen may have a certain role in the modification of the saliva. At least this RNP constitutes one of the component parts of the stylet sheath material which is secreted by the gland at the time of insect feeding[12]. Other roles of RNA in the form of RNP in the gland lumen remain to be investigated further.

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