ing gradients. A dissociating effect on hemoglobin was observed. This protein sedimented with an apparent molecular weight of 32,000 daltons, corresponding to one half of the molecule.

Essentially the same results were obtained with conventional sucrose gradients without polyacrylamide monomers.

In order to test the reproducibility of the method the following experiment was performed. Myoglobin, transferrin and IgG were chosen as 'unknown' proteins and ultracentrifuged in the presence of several standards. The molecular weights calculated from the plot were as follows: myoglobin, 18,700 ± 1500; transferrin, 81,200 ± 12,000 and IgG, 143,000 ± 16,000 (average of five independent determinations for each protein). A deviation of 8% or less was found when these values were compared with those reported for these proteins. The correlation and accuracy found is comparable with the results of most electrophoretical techniques to estimate the protein molecular weights. The linear relationship found may be easily justified if the variations in viscosity and density along the tube are neglected.

The plot proposed has some advantages in comparison with the procedure to estimate molecular weights described by Martin and Ames, because it allows not only the simultaneous use of a wide range of standards, but also the statistical fit of the data by linear regression.

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## Autoradiographic studies on RNA synthesis and transport in the ovary of *Hydrophilus olivaceus* Fabr. (Hydrophilidae, Polyphaga, Coleoptera)

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*Summary.* Autoradiographic studies using $^3$H-uridine in the ovary of *Hydrophilus olivaceus* Fabr. show that nurse cells, the germinal vesicle and follicular nuclei play an important role in contributing RNA whereby the major portion of RNA comes from the nurse cells.

*Key words. Hydrophilus;* telotrophic ovariole; RNA; nurse-cell; germinal vesicle; follicular epithelium.

Ribonucleoproteins are elaborated either by the nurse cells and trophic tissues or by the germinal vesicle[2,3]. In panoistic ovarioles the nucleolus plays an important role in contributing RNA to the oocyte whereas in meroistic ovarioles nurse cells are predominant[2]. Trophocyte nuclei have been shown by autoradiographic studies to be the main centers of RNA synthesis in a large number of polytrophic ovarioles, such as *Musca*[4-6], adephagous Coleoptera[7], *Panorpa*[8] and *Cecropia*[9]. In *Panorpa*[8] a major portion of RNA is supplied by the nurse cells but a smaller quantity also comes from the follicular epithelium. The Coleoptera form an interesting group where polyphagous Coleoptera generally possess telotrophic ovarioles while adephagous Coleoptera have polytrophic ovarioles. In the former, important variations occur[10-12]. In some species well defined nutritive strands may break at an early stage of development, so that the ovariole may appear to be panoistic. A modified telotrophic ovariole ('adenotrophic ') has been reported in *Steraspis speciosa*[13]; and another variant occurs in *Aulacophora foveicollis*[14]. Histological and histochemical studies of *Hydrophilus* ovary were made earlier[15]. A possible new type of ovariole ('mesotrophic') has been proposed depending on the absence of true trophic cords. Extrusion of ribonucleoproteins has been demonstrated histochemically but their origin and fate was not clear. Here we attempt to elucidate the question by autoradiography.

*Materials and methods. Hydrophilus olivaceus* Fabr. females were collected from seasonal fresh water ponds in Varanasi, India. To investigate RNA metabolism, the insects were injected with $^3$H-uridine (sp. act. 2.8 Ci/m mole; dosage 5 µCi/0.05 ml) and incubated for 15 min, 1, 2, 4 and 6 h. At the end of these incubation periods the ovaries were dissected and fixed in Carnoy's fixative. Paraffin sections were processed for autoradiography, using Kodak AR-10 stripping film. Exposure time varied from 6 weeks to 12 weeks. Appropriate RNAse controls for the autoradiographs were done. Semithin sections of the normal ovary were cut using an ultratome and stained with 1% toluidine blue to study its general histology.

*Observations.* Figure 1 shows the interrelationship between the component parts of the female reproductive system of *Hydrophilus*. Here each ovariole shows a terminal filament, germarium and vitellarium. In the immature females, the germarium is more than twice as long as the vitellarium. This can be divided into three zones. Zone I (apical) shows nurse cells having large spherical nuclei and a thin layer of cytoplasm. The middle zone (II) has larger nurse cells of polygonal shape. In this zone small cytoplasmic projections are seen coming out of the cells. The cytoplasm contains basophilic nucleoli. In zones

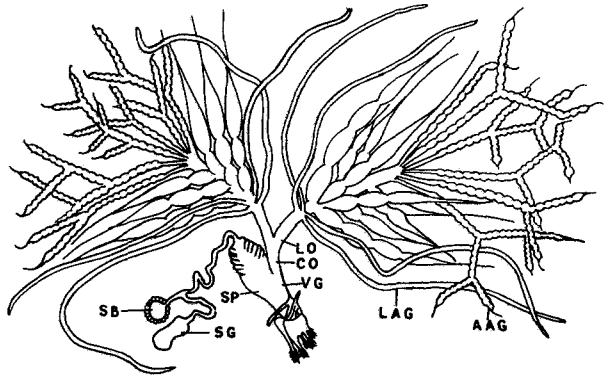


Figure 1. *Hydrophilus olivaceus*. Diagram of female genitalia. AAG, apical accessory gland. CO, common oviduct. LAG, lower accessory gland. LO, lateral oviduct. SB, spermathecal bulb. SG, spermathecal gland. SP, spermatheca. VG, vagina.

I and II binucleate nurse cells occur; sometimes two to four nurse cells show cytoplasmic connections between them (fig. 2). In zone II a few prefollicular cells are also seen scattered in between the nurse cells. Zone III shows somewhat smaller nurse cells, prefollicular cells and a limited number of developing oocytes which are designated as stage I oocytes. They contain a large germinal vesicle with diffuse chromatin and one or two nucleoli. When examined after different incubation periods, ovarioles show heavy labeling of nurse cells throughout the germarium (fig. 3–5). At 15 min incubation nucleoli of nurse cells show strong labeling, which increases further at 30 min. At 30 min cytoplasmic bridges between nurse cells show the presence of radioactivity (fig. 3). At 1 h incubation small cytoplasmic projections from nurse cells contain radioactivity (fig. 4). At 2 h radioactivity is seen in trophic cord-like projections in zone III (fig. 5). At 4 h nurse cells in this zone show strong labeling (fig. 6). This increases further with 6 h incubation.

The nucleoli present in the germinal vesicle of stage I oocytes show labeling (fig. 6). At 30 min of incubation nucleoli of stage II oocytes show radioactivity which is also visible in ooplasmic nucleolar extrusions (fig. 7). In stage III oocytes a strong labeling of nucleoli is seen at 1 h incubation. The anterior pole ooplasma is labeled (fig. 6 and 8). This is more clear in stage II oocytes.

The follicular epithelial nuclei are labeled in the nucleolar part after short incubation periods (fig. 7) but radioactivity spreads over the whole area of the cytoplasm at longer incubation (fig. 9). Follicular cells are tightly packed at earlier stages but become loosely arranged later. In histological preparations follicular cells show cyclical changes with the development of oocytes[15].

When the slides were treated with RNAse after incubation, it was found that radioactivity disappears from the nurse cells, its cytoplasmic cords, germinal vesicle and follicular cells suggesting thereby the complete removal of RNA molecules from these areas.

*Discussion.* The presence of radioactivity in nurse cells and in their cytoplasmic projections indicates that ribonucleoprotein particles elaborated by the nurse cells reach the oocyte, which is confirmed by the presence of radioactivity at the anterior pole of the latter. Such a flow of RNA was demonstrated in the egg-nurse cell complex of several Diptera[6] and Coleoptera[7]. The labeling of nucleoli in the germinal vesicle at short incubation periods and nucleolar extrusion bodies at longer periods suggests that a small amount of RNA is also being contributed to the ooplasm by the germinal vesicle. In the same way the follicular cells of *Hydrophilus* also seem to contribute, as is the case e.g. in *Panorpa*[8]. The change of shape of follicular cells plays a key role in the regulation of the flow of yolk precursors to the oolemma during oogenesis[16]. This may be the case also in *Hydrophilus* where a major portion of yolk protein is probably derived from the hemolymph[15], and another part endogenously synthesized by the oocyte. In *Ephestia kühniella*[17] part

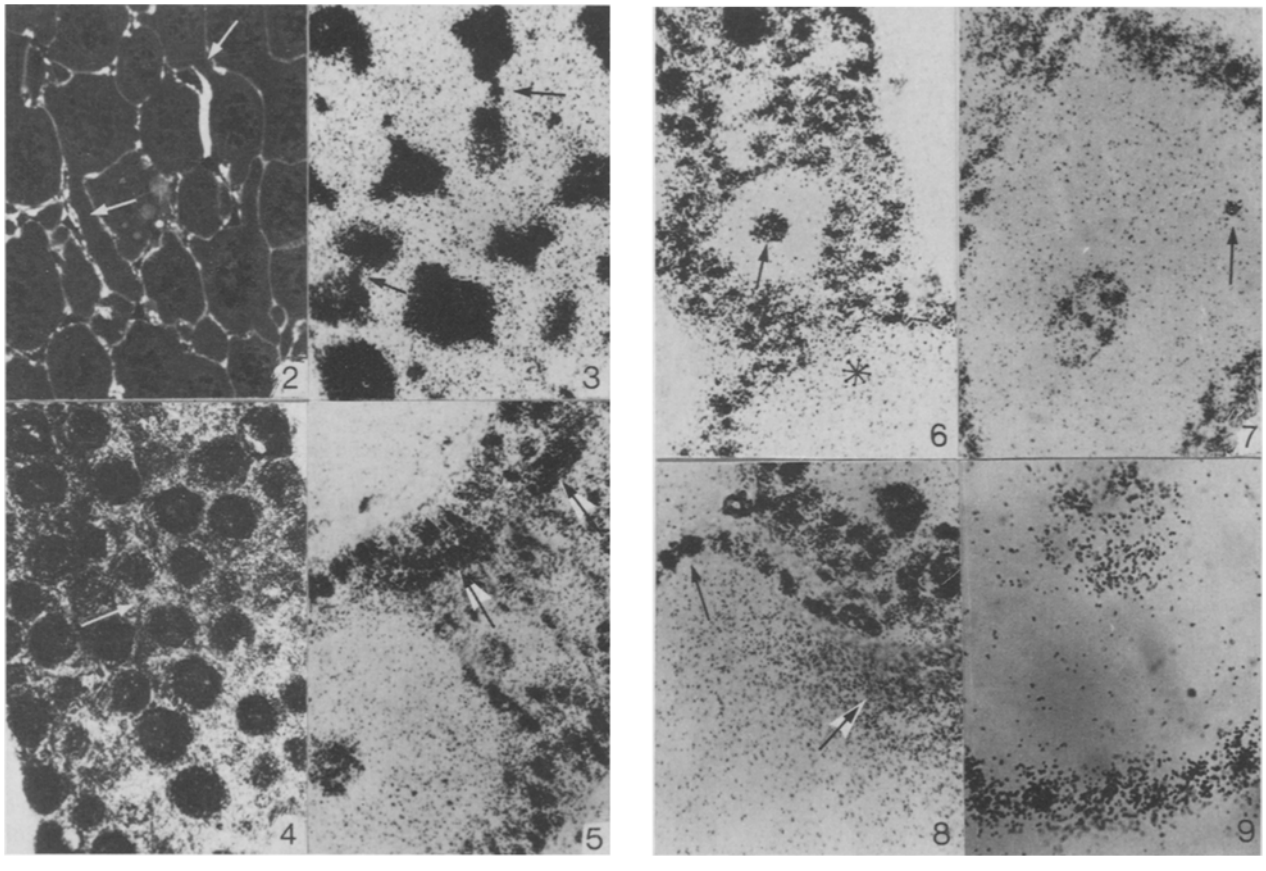


Figure 2–8 ( × 450), and 9 ( × 325): 2, Semithin section of germarium stained with 1% toluidine blue. Note the cytoplasmic connections between nurse cells (arrows). – 3, Nurse cell labeling at 30 min incubation. Besides the nuclei, cytoplasmic connections between cells (arrows), and basophilic nucleoli in the cytoplasm are labeled. – 4, At 1 h incubation, cytoplasmic projections of nurse cells are labeled (arrow), – 5, Transition zone of germarium after 2 h incubation. Note label in the oocyte nucleus (nucleoli), and in cytoplasmic cord which connects nurse cell with stage II oocyte (arrows). – 6, Transition zone at 4 h incubation. Label in nurse cell nuclei and cytoplasm, but also in the germinal vesicle (arrow) and anterior pole ooplasm (*) of stage II. – 7, stage III oocyte: nucleoli of the germinal vesicle and one extruded nucleolus are labeled after 30 min incubation. – 8, stage III oocyte with strong labeling in anterior pole ooplasm and in nuclei of the follicular epithelium. Incubation 1 h. – 9, stage II oocyte after 1 h incubation. The follicular epithelium shows diffuse labeling, and dense labeling of nucleoli.

of the yolk synthesized inside the ooplasma is initiated by follicular cells itself and another part comes from the hemolymph. In *Hydrophilus olivaceus,* the nucleolar origin of RNA and its stable nature suggest that this is rRNA which enters into the formation of yolk bodies, as described earlier for the ovary of *Musca* by Bier[4].

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## IgG purification to measure the level of an iodinated thyroglobulin peptide, the 3,5,3′,5′ tetraiodo-l-tyrosyl-l-tyrosine in human serum[1]

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*Summary.* Antibodies reacting with 3,5,3′,5′ tetraiodo-l-tyrosyl-l-tyrosine ($I_2$Tyr-$I_2$Tyr) were elicited in rabbits by immunization with an oxidized yeast conjugate coupled with $I_2$Tyr-$I_2$Tyr. Ion-exchange chromatography was used to purify immunoglobulins, in order to improve the specificity in measurement of $I_2$Tyr-$I_2$Tyr level in patient serum. IgG binding capacity versus $I_2$Tyr-$I_2$Tyr was considerably increased after immunoglobulin purification.
*Key words.* Thyroglobulin, iodinated; IgG purification; tetraiodo-l-tyrosyl-l-tyrosine.

$I_2$Tyr-$I_2$Tyr has been isolated from bovine thyroglobulin tryptic digests[3] and identified in thyroglobulin from other animals. Thyroglobulin, and some of the products of its proteolytic cleavage, are known to enter the circulating blood in some pathological thyroid diseases. Therefore, evidence was needed to determine whether or not $I_2$Tyr-$I_2$Tyr was actually present in the blood of euthyroidian and dysthyroidian subjects. To this end, we needed a precise and sensitive measurement for the presence of this compound in human serum and developed a technique of radioimmunoassay of $I_2$Tyr-$I_2$Tyr. The first attempts to produce $I_2$Tyr-$I_2$Tyr antibodies were carried out using oxidized yeast coupled with $I_2$Tyr-$I_2$Tyr, and were carried out in rabbits. This hapten-carrier immunogen was produced by the technique described by Bernard et al.[4]. After periodate oxidation, the yeast cell wall produces reactive aldehyde groups that react with side-chain amino group of $I_2$Tyr-$I_2$Tyr. The coupling is stabilized, and nonreactant aldehydes are blocked by reduction with sodium borohydride. The antisera thus obtained showed that this iodinated fraction of thyroglobulin is a normal constituant of human serum at a very low concentration. In order to determine its biological and physiological significance in human serum, it is necessary to purify the antisera. So the fractionation of immunoglobulin classes was performed to improve the measurement.

*Materials and methods.* $I_2$Tyr-$I_2$Tyr was synthesized and labeled with $^{125}$iodine with a sp. act. of 1200–1500 μCi/μg at INSERM U 71 by the technique described by Maurizis et al.[5]. The yeast *Saccharomyces cerevisiae* was supplied by Gallier Laboratories (Paris, France). Before oxidation, the yeast was washed with a 0.05 M phosphate-buffered saline (PBS) (pH 7.2) containing 0.1 M NaCl, then with a 0.2 M HCl glycine buffer (pH 2.5), and finally with PBS.

For coupling $I_2$Tyr-$I_2$Tyr to oxidized yeast, 0.5 g yeast was oxidized in 500 ml 1 N HCl containing 0.1 M sodium-periodate ($NaIO_4$) (Merck). The reaction medium was stirred for one hour at room temperature. The yeast was then centrifuged at 2000 × g, extensively washed with PBS, centrifuged and resuspended with a Potter-Elvehjem homogenizer in 10 ml of absolute ethanol. 20 mg $I_2$Tyr-$I_2$Tyr was incubated for 20 h with 0.5 g oxidized yeast in 10 ml of absolute ethanol. The coupled yeast was centrifuged at 2000 × g, washed with 0.1 N $NH_4OH$ to eliminate the unbound $I_2$Tyr-$I_2$Tyr, washed with $H_2O$ and then with 0.04 M barbital buffer (pH 8.4) and resuspended with a Potter-Elvehjem homogenizer in 500 ml of the same buffer. Reduction with 1% sodium borohydride ($NABH_4$) (Sigma) was carried out under magnetic stirring in 500 ml barbital buffer (pH 8.4), for one hour at room temperature. The yeast was centrifuged, extensively washed in PBS and resuspended with a Potter-Elvehjem homogenizer in 100 ml of the same buffer. The amount of $I_2$Tyr-$I_2$Tyr bound to yeast, after coupling, was 9.3 mg/g yeast[4].

Immunization. 10 New Zealand male rabbits were immunized. They received an i.v. injection of 5 mg of yeast coupled to $I_2$Tyr-$I_2$Tyr suspended in 1 ml PBS daily, for 5 consecutive days. The 5-day immunization cycle was carried out twice with a week-long rest period between each cycle. 1 month later, the rabbits received two booster injections of 5 mg yeast: an i.m. injection followed by an i.v. injection 6 h later. 1 week after the last injection, blood samples were taken.

Immunoglobulin fractionation was performed using DEAE-Sephacel (Pharmacia). Immunoglobulin fractionation[6] with DEAE-Sephacel, which separates IgG, IgA, and IgM classes was preferred to antibody affinity purification which separates only the IgG class from other serum proteins. 3 ml of rabbit serum previously dialyzed against PBS was eluted from DEAE-Sephacel with 30 ml of 0.0175 M phosphate buffer (pH 6.8), 40 ml of 0.08 M phosphate buffer (pH 6.6) and 40 ml of 0.3 M phosphate buffer (pH 6.5). The OD was measured at 280 nm in each eluted fraction. The top of each peak was concentrated to OD 280 nm = 10.

Binding capacity measurement. $5 \times 10^{-12}$ g of $^{125}I_2$Tyr-$^{125}I_2$Tyr (about 45,000 dpm) dissolved in 0.1 ml of 0.05 M phosphate