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## A new plot to estimate protein molecular weight by density gradient ultracentrifugation

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*Summary.* A plot of the logarithm of the molecular weight against the logarithm of the sedimenting distance is proposed for estimation of protein molecular weight. The proteins are separated in acrylamide-containing linear density gradients, polymerized and stained after centrifugation.
*Key words.* Protein molecular weight; molecular weight, protein; density gradient ultracentrifugation; ultracentrifugation, density gradient.

Sedimentation analysis in density gradients using the preparative ultracentrifuge is a standard method for protein characterization. This method allows the estimation of sedimentation coefficients and molecular weights of the samples by comparison with appropriate standards[1]. In a variant of the method, the sedimenting proteins are detected by means of the usual protein stains in acrylamide-containing density gradients, polymerized by photocatalysis after centrifugation[2].

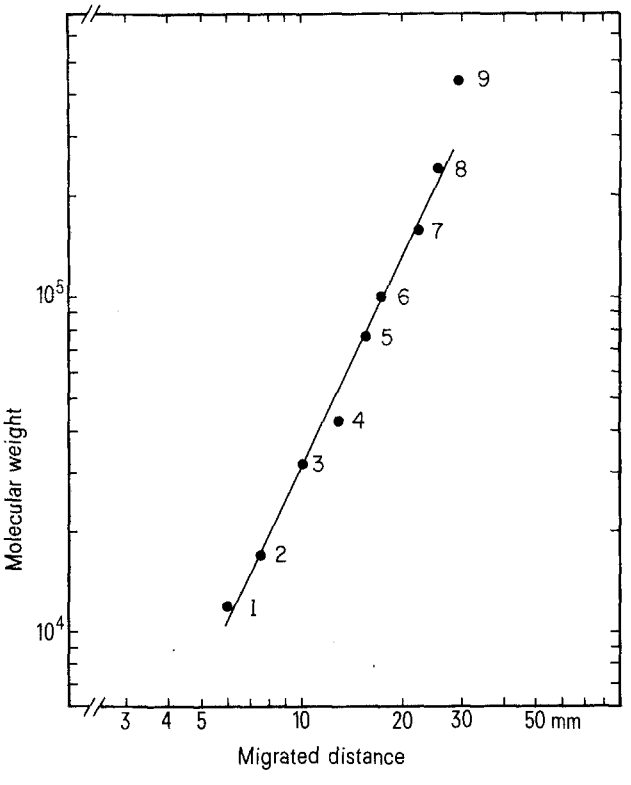
According to Martin and Ames, the position of the protein zones can be related to the corresponding sedimentation coefficients, which can be used to estimate molecular weights[1].

The plot that we propose is an alternative procedure for determining protein molecular weights, using linear sucrose density gradients in the presence or absence of acrylamide.

The acrylamide-containing density gradients, and the gel formed, were similar to the ones previously reported[3], with only slight modifications. Briefly, linear density gradients of sucrose from 5 to 30% (w/v) were formed in 5 ml cellulose nitrate tubes. These tubes contained 50 mM Tris-HCl buffer pH 8.8, 8% acrylamide, 0.3% N,N-methylene bis acrylamide, 0.2% N,N,N′,N′-tetramethylethylendiamine and 6 µg/ml riboflavine. Centrifugation was carried out in a Beckman L5-65 preparative ultracentrifuge with SW-50.1 rotor for 15 h at 300,000 × g. The tubes contents was polymerized by exposure to a long-wave UV source. The gels obtained were removed from the tubes by injecting water between gel and tube wall, and stained with Coomassie brillant blue G-250. The amount of protein detectable in each band under these conditions ranged from 10 to 100 µg. The sucrose density gradients without acrylamide monomers were also from 5 to 30% sucrose, in the same buffer.

The position of each band was measured directly with a millimeter rule from the top of the gel to the center of each band. The logarithm of molecular weight for each protein was plotted against the logarithm of the distance. In the case of conventional gradients without acrylamide, the abscissa was the logarithm of the number of the fractions collected counted from the starting radius.

As shown in the figure, a linear relationship was found for proteins from 12,000 to 240,000 daltons in acrylamide-contain-




Linear relation between the logarithm of the molecular weight and the logarithm of the migration distance for several proteins. Standards are: 1, horse heart cytochrome c (Mr 11,700); 2, horse muscle myoglobin (Mr 17,200); 3, half human hemoglobin (MR 32,000); 4, chicken egg albumin (Mr 44,600); 5, human transferrin (Mr 78,000); 6, yeast hexokinase (Mr 102,000); 7, rabbit muscle aldolase (Mr 160,000); 8, bovine liver catalase (Mr 232,000); 9, horse spleen ferritin (Mr 440,000). Each point represents the mean obtained from 12 independent experiments. The straight line was obtained by the method of least squares. (Excluding ferritin: r = 0.995.)

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

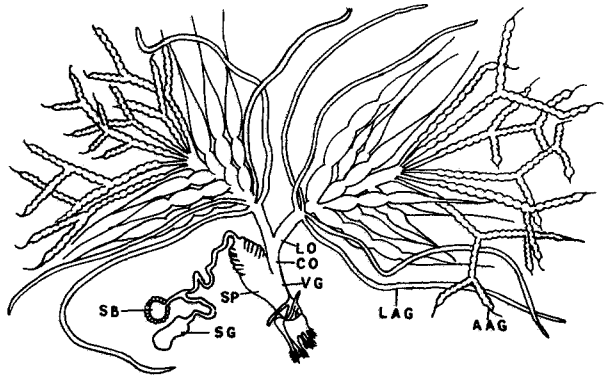


Figure 1. *Hydrophilus olivaceus*. Diagram of female genitalia. AAG, apical accessory gland. CO, common oviduct. LAG, lower accessory gland. LO, lateral oviduct. SB, spermathecal bulb. SG, spermathecal gland. SP, spermatheca. VG, vagina.