An Electron Microscope Study of the Anal Organs of *Drosophila* Larvae

In a previous study GLOOR and CHEN[1] demonstrated the occurrence of a pair of anal organs located ventrally at the posterior end of *Drosophila* larvae. When the larvae were briefly immersed in a 0.5% $AgNO_3$ solution and shortly exposed to light, two symmetrical dark-brown colored plates could be observed, due to the deposition of silver granules in the cuticle and the hypodermal cells. Otherwise it is difficult to notice these plates because of their similar appearance as the surrounding body parts. From their physiological analyses GLOOR and CHEN concluded that these organs absorb inorganic ions such as chloride and sodium from the hypotonic media.

The anal organs are also of interest in view of their possible significance in the lethal mutation *l(3)tr*. As pointed by HADORN[2] and CHEN[3] the enormous accumulation of body fluid in the mutant larvae could causally be related to a disturbance of the osmoregulatory mechanism. In fact, the osmotic concentration of the hemolymph of *ltr* larvae has been found to be only about 50–60% of that of the wild type (BRUGGER, unpublished). For this reason we carried out a detailed study of the fine structure of these organs, and their functional-morphological relationships between the wild type and the lethal homozygotes were compared. The detailed results of this study will be published later.

Drosophila melanogaster larvae of both genotypes were raised on standard medium at 25 °C. At the desired stage the larvae were immersed for 1 h in either distilled water or hypertonic solutions containing 1.5 to 10% NaCl. Animals removed directly from the culture medium served as controls. The posterior tip of each carefully washed larva was cut in a drop of fixative containing 3.2% acrolein and 1.7% glutaraldehyde in cacodylate buffer (0.5 *M*, pH 7.2). After 1 h at 4 °C the piece was washed for 3–4 h in cacodylate buffer whose osmotic concentration was adjusted with a 10% sucrose solution. Following this, it was post-fixed in buffered 1% OsO_4 for 2–3 h at room temperature, washed again for 5–6 h, dehydrated in graded alcohol series and embedded in araldite. Sections were cut with a Porter-Blum microtome and mounted on carbon-coated copper grids. The sections were then stained in saturated 70% alcohol solution of uranyl acetate and lead citrate (REYNOLDS[4]). A Hitachi HS-8 electron microscope was used for observation.

For scanning electron microscopy whole larvae were fixed on a slide with scotch tape, immediately frozen in liquid nitrogen (−196 °C), and lyophilized for 12 h.

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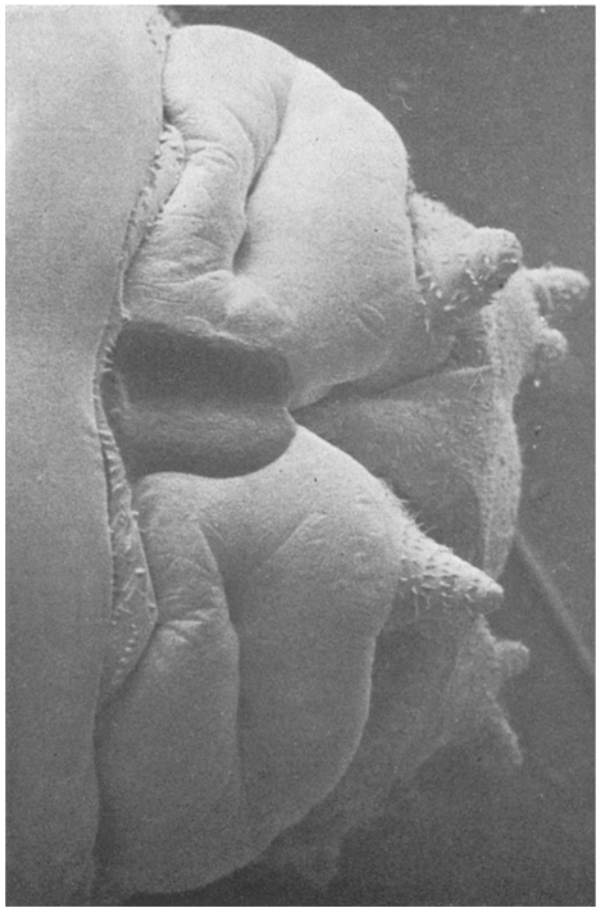

Fig. 1. Scanning electron micrograph (SEM) showing the posterior-ventral view of a symmetrical pair of anal organs of a 6-day-old *ltr* larva. ×640.

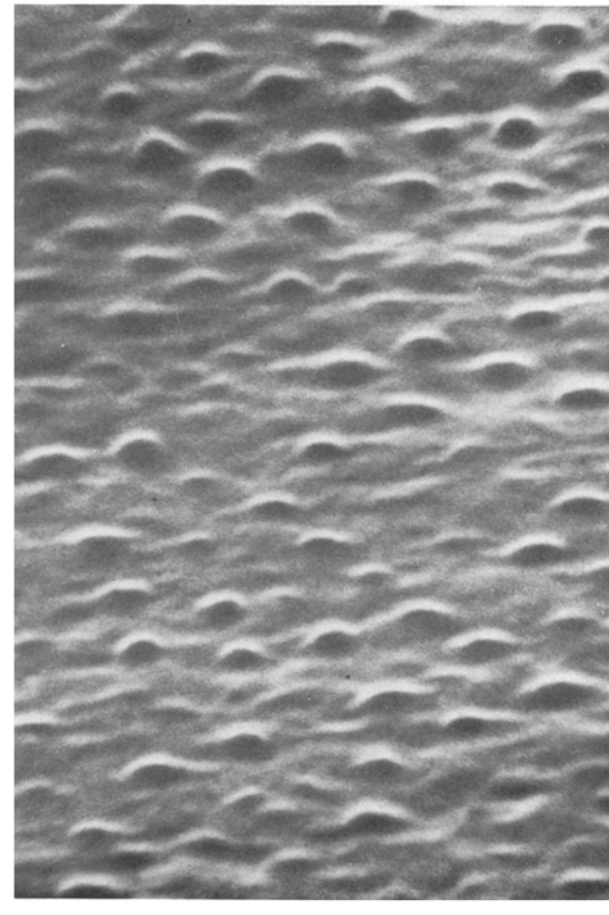

Fig. 2. Porous appearance of the cuticular surface of the anal organ (SEM), ×42,300.

Subsequent to coating with gold these were studied with a surface scanning electron microscope (Cambridge Stereoscan S4).

Figure 1 is a scanning electron micrograph of the two symmetrical anal organs of a fully grown *ltr*-larva under normal culture condition. As can be seen, the two organs nearly contact each other posterior to the anus, but are widely separated anterior to it. In the central part of each organ there is a transverse slit-like depression. Our experience showed that both organs appear distinctly elevated in a hypotonic medium (distilled water) and become more flattened in a medium of high salinity (5.85–10% NaCl). This indicates that there is an increase of the surface area with an increasing demand of ions from the environment.

Under high magnification it has been found that the cuticular surface of the anal organs, in contrast to the surrounding cuticular parts, has a porous appearance (Figure 2). However, our detailed analysis of the sections revealed no pore formation in the anal organs and their porous appearance is in fact due to infoldings of the epicuticle. Such an arrangement obviously leads to a further increase of the surface area of the overlying cuticle to facilitate ionic absorption.

In agreement with the earlier finding of GLOOR and CHEN[1], the present electron microscopic observation of thin sections (500 to 700 Å) also showed that in the anal organ region the cuticle is thinner and the hypodermal cells are much larger than those in the remaining body parts. Each organ is separated from the neighbouring exoskeleton by a cuticulin layer. Sections from animals briefly treated with $AgNO_3$ solution demonstrated that the silver granules were first located in the epicuticle and later moved into the endocuticle.

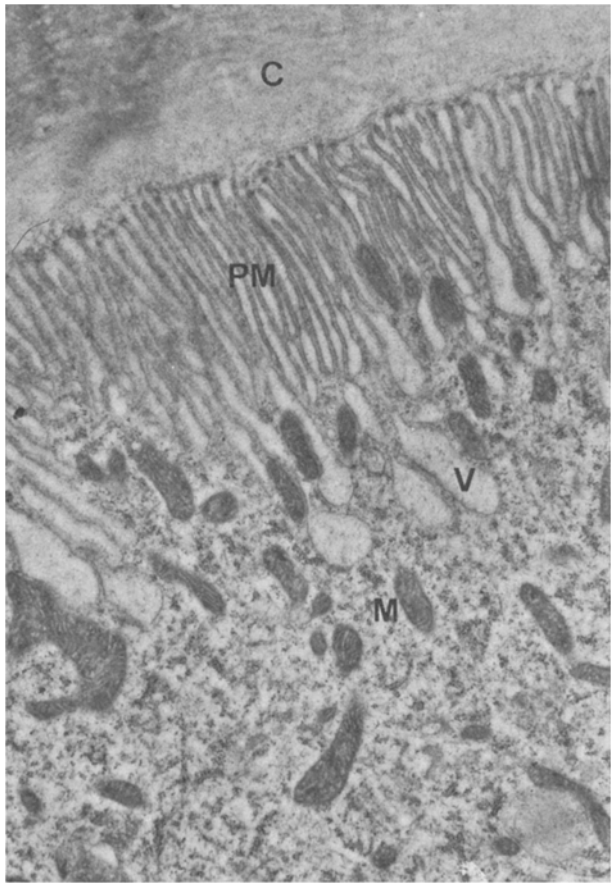

Fig. 3. Transmission electron micrograph of a cross section of a hypodermal cell in the anal organ of a mature wild-type larva. C, cuticula; PM, plasma membrane; M, mitontodria; V, vacuole. ×16,300.

As illustrated in Figure 3, on the cuticular surface the plasma membrane of the hypodermal cells forms numerous folds which are oriented perpendicular to the cuticle. Between the folds interspace of varying sizes can often be observed. In contrast, the plasma membrane on the basal surface appears quite smooth. In the cytoplasm of the hypodermal cells there are numerous, randomly distributed mitochondria. Under normal condition these rarely lie between the folds of the plasma membrane (see Figure 3). The cells have a single large, usually ovoid nucleus. Free ribosomes are abundant, but endoplasmic reticulum is sparse. Vacuoles occur more frequently in the cytoplasmic region near the apical folds, from which they seem to be constricted. These organelles also appear scattered in the central and basal region of the cell. Microtubules which run throughout the cytoplasm show often direct connections to small rounded vacuoles. The latter are also visible between the infoldings of the plasma membrane on the cuticular surface. Lysomes are rare and have occasionally myelin-like inclusions. Glycogen has been identified but, peculiar enough, Golgi material is very rarely noticed.

Of special interest is the profound alteration in the fine structure of hypodermal cells by exposing the larvae to solutions of different salinities. In a hypotonic medium (distilled water) the folds of the plasma membrane on the cuticular surface become increased and penetrate more deeply into the cell. The number of mitochondria is also increased and many of them are located between the apical folds, whereas this has rarely been seen in larvae removed directly from the culture medium (Figure 3).

An entirely opposite picture was found in animals treated for 1 h in a hypertonic solution containing 1.5% NaCl. The folds of the plasma membrane become shorter and fewer in number and assume an irregular arrangement. By increasing the salinity to 5.85% the folds can nearly be no more recognized. The mitochondria are greatly reduced and none is visible between the folds. Many of these appear even vacuolized. Vacuoles of heterogeneous sizes with various inclusions, including vesicles 0.05–0.1 μm in diameter, become increasingly numerous. Furthermore, multivesicular bodies, unidentified electron density and lamellate structures have been observed.

In general, our results demonstrate that the ultrastructure of the anal organs in *Drosophila* larvae bears a great similarity to that of the anal papillae in the mosquito larvae[5,6]. The ion uptake process through the anal organs can obviously be effected by varying the surface area of the plasma membrane on the cuticular surface of the hypodermal cells. The marked increase in the number of mitochondria as well as the migration of these organelles into the interspace between the folds are also in agreement with the view that the ionic absorption must be an energy-consuming process.

Contrary to our expectation, we have so far found no detectable difference in the fine structure of the anal organs between the wild type and the *ltr* larvae. If osmotic regulation is really involved in the lethal mutation, the effect must occur at some other levels. A more detailed analysis of the functional aspect of these organs would prove to be profitable.

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Zusammenfassung. Elektronenmikroskopische Untersuchungen des Analorgans bei Drosophilalarven zeigten, dass die cuticulare Oberfläche dieses Organs durch Einstülpungen der Epicuticula vergrössert wird. Die an die Cuticula angrenzende Plasmamembran der Hypodermiszellen bildet zahlreiche Faltungen. Je nach der Salinität des Aussenmediums weist die Anzahl der Plasmamembranfaltungen und der im Cytoplasma lokalisierten Mitochondrien regulatorische Veränderungen auf.

[7] This work was supported by grants from the Schweizerischer Nationalfonds and the Georges und Antoine Claraz-Schenkung.

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Accumulation of Ribonucleoprotein Structures in Chronically Virus-Infected Cells

A line of HEp2 cells chronically infected with tick-borne encephalitis (the strain Sofyin) has been obtained which was designated HEp2-Sof[1]. This culture is characterized by a retarded cell growth, the resistance to superinfection and the release into the medium of infectious material in a low concentration[2]. We describe in this paper virus-specific structures revealed in this system.

Experiments were conducted with the HEp2-Sof cell line, which within 13 years, has undergone about 400 passages. In 5 to 7 days after reimplantation into medium 199 with 10% bovine serum, a monolayer was formed, which was used for the study. Actonomycin D (5 μg/ml) was added for inhibition of the synthesis of cellular RNA: in the control non-infected HEp2 culture, the RNA synthesis was inhibited to 97–99%. Newly synthesized viral RNA in the HEp2-Sof cells was labelled by introduction of ^{3}H uridine (5 μCi/ml, specific activity 1.5 Ci/mmole), 2 h after the antibiotics had been added, for 5 to 6 h. At the end of this period the culture was rapidly chilled and washed with the cold Hanks' solution. The cells were collected mechanically into STE buffer (*Tris* HCl 0.01 *M* pH 7.4, NaCl 0.1 *M*, EDTA 0.001 *M*), washed in a centrifuge (1200 × *g*, 10 min), resuspended in RSB (*Tris* HCl 0.01 *M* pH 7.4, NaCl 0.01 *M*, $MgCl_2$ 0.0015 *M*), the cells were disrupted in a Dounce homogenizer, the nuclei were removed by centrifugation (1200 × *g*, 10 min) and cell homogenates thus obtained were studied.

Figure 1 presents sedimentograms of virus-specific structures revealed in sucrose density gradients. It is seen that these structures sediment with the fraction of mitochondria and membranes and are separated from them by the treatment of the cell homogenates with a non-ionic detergent (NP40), being non-sensitive to the EDTA treatment. Therefore these structures are true ribonucleoproteins, but not polyribosomes. Two types of ribonucleoproteins are revealed according to their sedimentation properties, having the sedimentation coefficients 180 S and 140 S. It is worthwhile to note that Sendai virus ribonucleoprotein, labelled with P^{32}, was used as a marker, whose sedimentation coefficient is known to be 210 S[3]. In the given conditions of centrifugation, the fraction of mitochondria and membranes occupies an equilibrium position that corresponds to fraction with the density 1.18 g/ml, i.e. 50% sucrose, while the structures with the density more than 1.24 g/ml (60% sucrose) sediment and do not occupy the equilibrium position.

To determine the buoyant density of ribonucleoprotein structures, both peaks with the sedimentation coefficients 180 and 140 S were collected, fixed in 8% formaldehyde

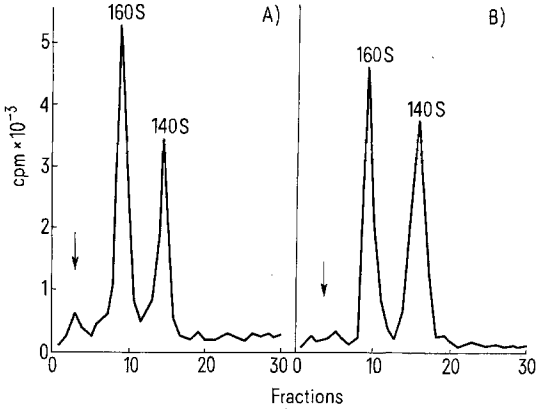

Fig. 1. Sedimentigrams of homogenates of HEp2-Sof cells after centrifugation in sucrose density gradients 15/60% in a rotor SW 27.1 of a Spinco L3 centrifuge at 22,500 rpm for 1 h 45 min. Conditions of the experiment are described in the text. A) non-treated cell homogenate; B) treated with 0.5% NP40 and 0.03 *M* EDTA. The position of the marker peak of P^{32} Sendai virus labelled ribonucleoprotein is shown by arrows.

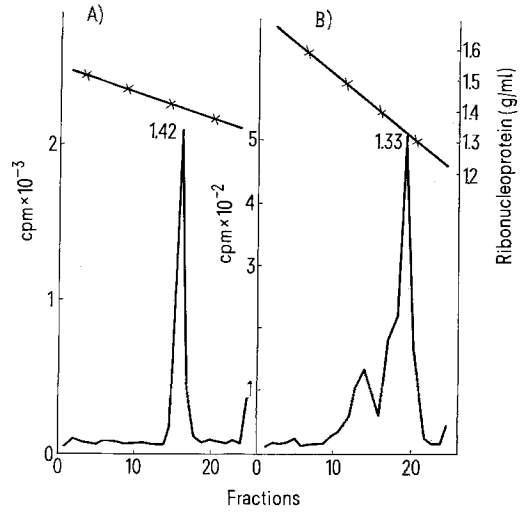

Fig. 2. Density distribution of ribonucleoproteins after equilibrium centrifugation in Caesium chloride density gradients in a SW50 rotor of a Spinco L3 centrifuge at 45,000 rpm for 3 h. A) sucrose gradient fractions that contain 180 S ribonucleoprotein; B) sucrose gradient fractions that contain 140 S ribonucleoprotein.

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