Salt Transport Organelle in Artemia salenis (Brine Shrimp)

Abstract. *The branchiae of* Artemia *adapted to triple-strength sea water (105 per mil salinity) were studied with the electron microscope. The epithelial lining of the metepipodite segment possesses organelles composed of stacks of disc-shaped mitochondria interlaced with flattened extensions of a canalicular system that in turn communicates with the plasma-bound surface of the cells. The distance between the canalicular and mitochondrial membranes is small and quite constant. The marked similarity to "mitochondrial pumps" in the anal papillae of mosquito larvae suggests that the organelle is concerned with salt transport.*

Aquatic organisms rarely have internal tonicities that are equal to the tonicity of their environment. Marine forms are usually hypotonic in relation to the environment, and freshwater forms almost invariably are hypertonic. The former condition requires export of salt and import of water. The latter condition reverses the relationship.

The fine structure of the epithelium in the anal papillae of a mosquito larva (*Culex quinquefasciatus*) has been described (*1*). The larvae were raised from egg raft to fourth instar in distilled water, which contained only traces of salt derived from the food given to the larvae.

At the end of the experiment the total salinity was 0.009 part per thousand. Thus, the epithelium that lined the papillae was under strong physiological demand to fulfill its function of absorbing salts (*2*) from dilute solutions. Characteristic of the cells were organelles labeled as "mitochondrial pumps" (*1*). They consisted of canalicular extensions, from the plasma surface of the cell, sandwiched in a very symmetrical fashion between pairs of flattened, pancake-shaped mitochondria.

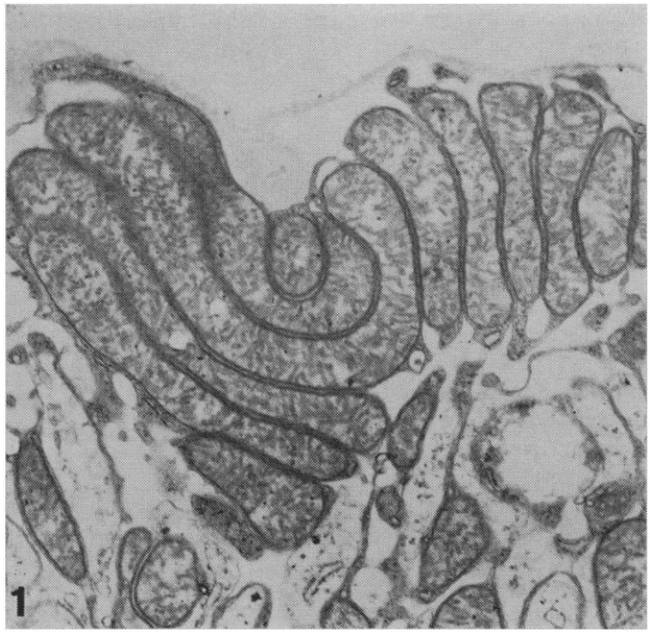
The same cellular organelle in a highly developed state in an organism at the other extreme of the tonicity relationship is described here. *Artemia salenis* were raised in triple-strength sea water from eggs to mature, breeding adults. Branchial elements were fixed in 5 percent glutaraldehyde buffered with Millonig's phosphate buffer to which 15 percent sucrose was added. The tissue was embedded in Epon. Part of the electron microscope observations were made on an RCA-EMU-3F, and part were made on a Siemens Elmiskop I.

The metepipodite segments of the branchiae were examined. These react to silver nitrate (*3*) and are believed to be responsible for salt transport (*4*). The segments are lined by a simple epithelium of two cell types. Stellate cells with dark cytoplasm surround and interdigitate in a complicated fashion with cells containing light cytoplasm (*5*).

Figures 1 and 2 show mitochondrial pumps that are highly developed in the dark cells. As in the cells of the anal papillae of mosquito larvae (see *1*), a meshwork of irregular canalicular spaces extends inward from the distal or plasma-contacting surface of the cells. Again, as in the mosquito larvae, flattened and parallel surfaces of the canalicular profiles are sandwiched between flattened mitochondria.

However, in *Artemia* the organelle consists of numerous mitochondria aligned like a stack of coins, each mitochondrion sharing a canalicular space with the adjoining one. Ten or 12 is not an unusual number and as many as 21 mitochondria have been counted in one organelle.

The spacing between the units of the organelle is quite constant (Figs. 1 and 2). The mitochondria may be pleomorphic, assuming curved or "S" shapes. In this case, however, adjoining mitochondria will conform to the same pattern, preserving the regular



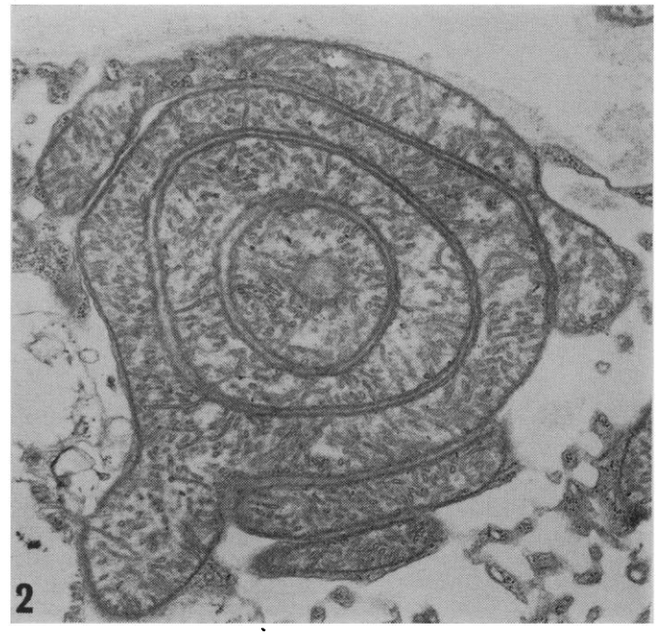


Fig. 1. Mitochondrial "pump" sectioned in a dimension at right angles to the one in Fig. 2. The plasma-bound surface is at the top (× 12,000). Fig. 2. Mitochondrial "pump" sectioned in a dimension at right angles to the one in Fig. 1 (× 12,000).

dimensions of the spacing between them.

Figures 1 and 2 illustrate a fortuitous observation made in the same section of material. Figure 1 is the more commonly observed arrangement of mitochondria, except that the mitochondria are unusually long and curved in at least part of the organelle. Figure 2 shows a most unusual concentric inlay of doughnut-shaped mitochondria. This apparent aberrant condition receives explanation if it is compared to the configuration in Fig. 1. The flattened mitochondria have assumed the shape of a series of nested cups. In Fig. 1 the "cups" have been sectioned from top to bottom, and in Fig. 2 they have been sectioned across their diameter.

The angle of section in relation to a particular set of membranes can be gaged by the sharpness of the profile (right angle) or the blurriness (less than right angle). With this in mind, it is obvious that the mitochondria have a relatively constant thickness in the dimension from one canalicular surface to the other. However, it is difficult to judge the longer dimension or diameter of any one pancake-shaped mitochondrion. There is probably considerable variation.

As in the case of the mosquito larvae (*1*) the spacing between the mitochondrial membranes and the canalicular membranes is relatively constant, as is also the dimension of the slot-like canalicular space. The rather unusual pancake-like dimensions of the mitochondria, together with the close tolerances of the membrane spacings, gives support to the idea that the structures constitute a cellular organelle that may participate in the metabolic transport of salt.

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References and Notes

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Restoration of Gamma Globulin Production in Agammaglobulinemic Chickens

Abstract. *Chickens irradiated and bursectomized in the newly hatched period consistently develop agammaglobulinemia and form no circulating antibodies; if the birds are treated immediately after operations by intra-abdominal injection of unirradiated autologous bursa cells, immunoglobulin production, lymphoid germinal centers, and plasma cells are restored; however, the birds fail to produce antibody to specific antigenic challenge.*

Glick demonstrated that surgical removal of the bursa of Fabricius of young chickens prevented development of normal antibody-producing capability (*1*), a deficit later produced by treatment of the embryo with testosterone and other progestational hormones (*2, 3*). These immunologically deficient chickens generally have germinal lymphoid centers and plasma cells in the splenic and intestinal lymphoid tissues, but have reduced amounts of circulating immunoglobulins (*3–6*).

Experiments in this laboratory have led us to a definition of the immunoglobulin-production system as a system of cells which apparently originates from and is dependent on the bursa of Fabricius (*7*), and includes as well germinal center cells and plasma cells. Bursectomy and nearly lethal total-body irradiation at hatching prevents development of germinal centers and plasma cells and results in agammaglobulinemia and complete failure of antibody production. This group of morphologic and functional characteristics resembles the sex-linked recessive agammaglobulinemia described by Bruton (*8*).

Attempts to provide bursectomized-irradiated newly hatched chickens with the missing cell system involved administration of their own bursal cells in the experiments reported here. Germinal centers, plasma cells, and γ-globulin production were restored; but, unexpectedly, significant amounts of agglutinating antibodies to both bovine serum albumin (BSA) and whole *Brucella* organisms were not produced.

Newly hatched white Leghorn chickens were randomly divided into three experimental groups. Bursae were extirpated immediately from two groups. These groups and an unoperated control group were irradiated with 740 roentgens in air. Bursal-cell suspensions were prepared by mincing the extirpated bursa in tissue culture medium 199 containing penicillin (100 unit/ml) and streptomycin (100 μg/ml) and then further disrupting the cells in a loose-fitting glass homogenizer. The cell suspensions were allowed to settle in an upright hemagglutination tube (10 minutes in an ice bath). The supernatant containing mainly lymphoid cells (Fig. 1) was removed, concentrated by centrifugation (4°C) for 10 minutes at 1000 rev/min, and the cells were resuspended in 0.5 ml of the original media prior to intra-abdominal injection into chickens of the bursectomized-irradiated group.

Animals surviving at day 40 were injected intra-abdominally with 20 mg of crystallized BSA (Armour) in saline and 10^9 killed *Brucella abortus* organisms (U.S. Dept. of Agriculture). Nine days later the birds were bled and killed. The spleen and the cecal

Table 1. Presence of germinal centers and plasma cells in the spleen or cecum and circulating 19*S* and 7*S* γ-globulins in experimental chickens. (Results are expressed as the ratio of responses to total number.)

Germinal centers	Plasma cells	γ-Globulins	
		19*S*	7*S*
Control-irradiated			
6/6	6/6	6/6	6/6
Bursectomized-irradiated			
0/6	0/6	0/6	0/6
Bursectomized-irradiated injected with bursal cell suspension			
7/7	7/7	7/7	7/7

Table 2. Primary antibody responses to *Brucella abortus* and bovine serum albumin in experimental chickens.

B. abortus		BSA	
Response/ total	Mean titer ($\log_2$)	Response/ total	Mean titer ($\log_2$)
Control-irradiated			
6/6	7.2	6/6	6.2
Bursectomized-irradiated			
0/6	0	0/6	0
Bursectomized-irradiated, injected with bursal cell suspension			
0/7	0	0/7	0