

by the regulation theory<sup>4</sup>. Figure 2, b shows the reaction of the system following a rectangular ascent and figure 2, c the reaction following a pulse in substrate supply.

Figure 2, d and e show typical differences between closed and open systems. After a pulsed substrate supply in the closed system (figure 2, d) the extinction decreases accordingly, which means a rise in product concentration in an accumulative manner. Figure 2, e shows the same pulse sequence in an open system.

After each pulse the concentration of product rises, but afterwards it approaches the equilibrium value again. Figure 2, f shows the linearity of the arrangement by applying linearly increasing quantities of substrate.

The results show that the arrangement renders accurately various flowing equilibria in a short time interval. This enables one to detect the optimal and limiting states of equilibria by simulation. As distinguished from a closed

system, self regulation of an open system by feedback of recognized data is provided. Especially in more complex systems like allosteric enzymes or organelles and cells the validity of this procedure is evident<sup>2</sup>. The figures also show that the accuracy and reproducibility of the arrangement are sufficient. Results concerning further experiments with allosteric enzymes and cells will be reported subsequently.

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## Ciliary locomotion in squid hatching

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**Summary.** Squid hatchlings are shown to use their transitory set of integumental cilia as a locomotory organ when they cross the gelatinous envelopes of the eggs.

All cephalopod hatchlings use a special gland to open the egg case. This 'organ of Hoyle' is known to produce an enzyme, and to stock it until it is released to dissolve the egg case locally<sup>2</sup>. Rather little is known of the auxiliary equipment that hatchlings use in working themselves out through the hatch opening.

Clearly the structure and functioning of the entire hatching equipment must be correlated with the nature of the egg case, which is a variable feature among cephalopod orders or sub-orders. Thus, in the incirrate octopods, the embryo is surrounded only by the so-called chorion and during hatching is assisted (if it is not using its arms) by a set of integumental hard structures; these are the 'Kölliker tufts' in their closed state<sup>3</sup>.

Among the decapods, the sepioids enclose individual eggs in gelatinous envelopes, which they wrap spirally around the chorion<sup>4</sup>. As the chorion increases considerably in size during embryonic development, the surrounding gelatinous layers are stretched and they become thinner. Thus the hatchling has to cross only 1 compound envelope. The teuthoids or squids, on the other hand, enclose several to very many eggs in one and the same envelope, which they wind on in the fashion of a spiral staircase<sup>5</sup>. In *Loligo vulgaris* and some other loliginid species, the chorion swells so strongly, and the outer envelopes become so thin that at the end of embryonic development the eggs show up as densely packed vesicles facing the open water with their bulging outer wall. Nevertheless, the greater part of the chorion is surrounded by the gelatinous layers separating one egg from another, so that the hatching animal may not immediately emerge into the open water. In other loliginids, such as *Alloteuthis*, the gelatinous envelope is still very thick all around the eggs at the end of embryonic development. This occurs even in *Loligo* egg masses infested by capitellid polychaetes<sup>6</sup>.

The question then is: how do the hatchlings cross gelatinous masses when funnel jetting does not have a locomotory effect? The answer is that the kinocilia of the animal's integument, which beat headwards, give the animal backward locomotion in the jelly that is liquified by the enzyme

emanating from the hatching gland. The amount of enzyme available is sufficient to make long ducts in the envelopes. To test this, animals were taken from their chorion when they were ready to hatch; thus the hatching gland remained intact. In a pipette with a tip sufficiently wide for a smooth passage, each animal was taken up and then injected, tail first, into the envelopes of an egg mass. Regularly the following events were then observed.

After a period of immobility lasting between a few sec and 1 min or more, the animal stretches out its mantle tip. This movement is known to initiate normal hatching<sup>7</sup>. It apparently causes the rupture of the apex of the gland cells, which liberates the enzyme. Immediately after the first 1 or 2 stretching movements, the animal begins to glide along, tail first, and steadily advances until it emerges at the surface of the egg mass (figure 1). There it immediately begins to swim by jetting. Inside the gelatinous envelopes, sporadic mantle contractions have no locomotory effect at all.

The cilia providing locomotion have been known before (apparently non-motile cilia are also present in the integument<sup>8</sup>). Ranzi<sup>9</sup> has described the currents of the perivitellinic fluid that are generated by the kinocilia of the embryo and its outer yolk sac. They all beat in anterior direction. The length and external structure of these cilia varies over different areas of the surface, and their arrangement differs particularly between the mantle and the head<sup>10</sup>. The dorsal and the ventral surfaces of the mantle of *Loligo* hatchlings show longitudinal strips of densely set cilia continuous over several cells (figures 2 and 3). Live observations show that in moribund hatchlings these cilia continue to beat steadily when the longer cilia of the separate ciliary cells on the head have already ceased to beat.

Although the mantle cilia, and particularly those arranged in the longitudinal strips, would appear to be the most effective in locomotion, the cilia of the head also have a part in it, as can be seen when the mantle of a hatching animal has broken through the surface of the egg mass (figure 1, e, f). Possibly these longer cilia are more effective in a fluid medium, whereas the shorter cilia of the mantle may have their greatest propulsive effect in a more viscous

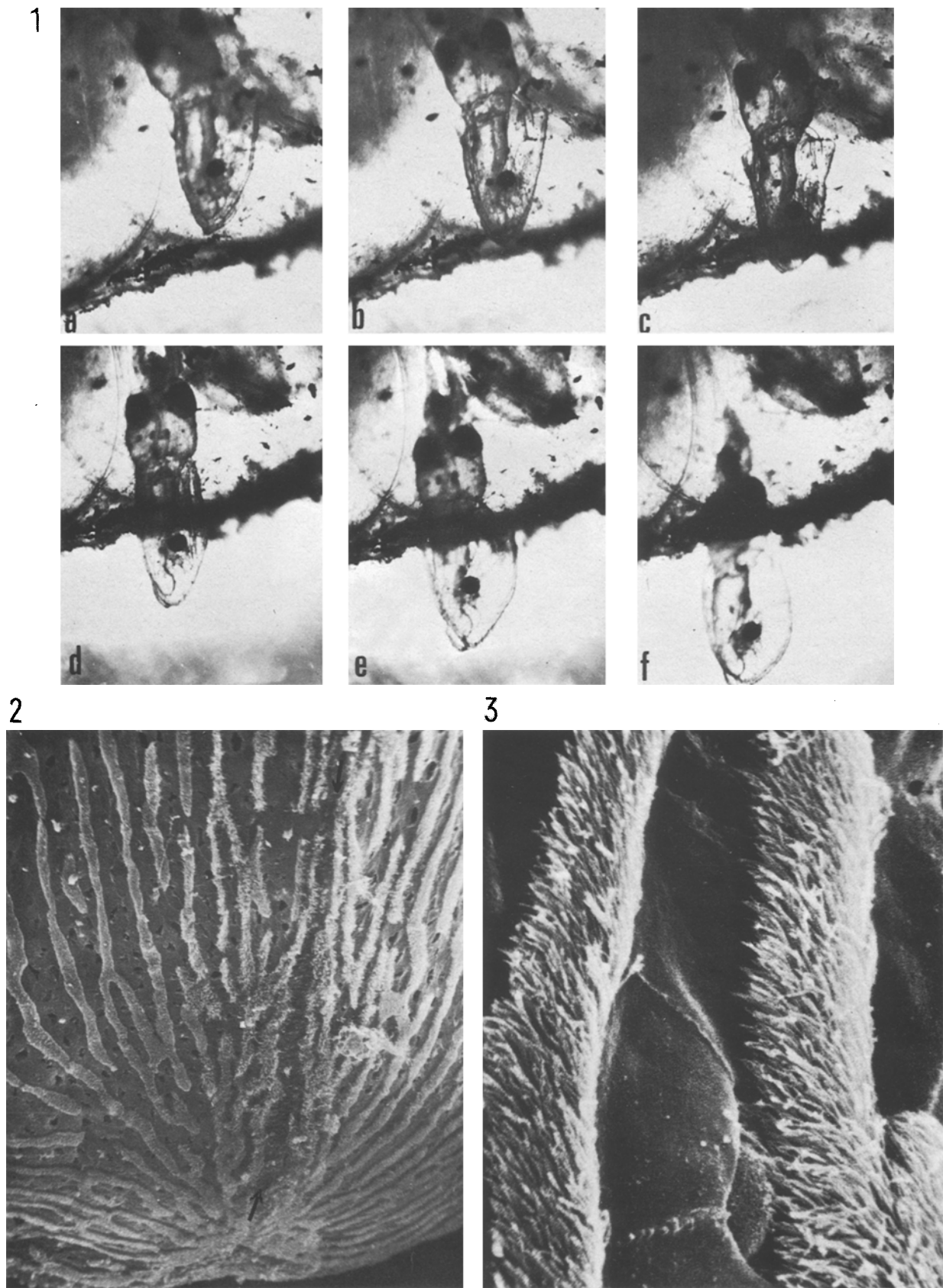


Fig. 1. Hatchling of *Loligo vulgaris* crossing the gelatinous envelopes of the egg mass by ciliary locomotion. Pictures have been taken at 3-4 sec intervals.  $\times 10$ . Fig. 2. Scanning electron micrograph of the dorsal side of the mantle end, showing the long ciliary bands. Arrows mark the line of the mid-dorsal limb of the anchor-shaped hatching gland.  $\times 250$ . Fig. 3. Ciliary bands in the anterior part of the dorsal mantle surface, at higher magnification.  $\times 2500$ .

medium and on nearly solid substrates. The mantle end indeed represents the advancing head of a 'borer', around which the jelly is only beginning to liquify, whereas the animal's head and arms trailed behind have their cilia beating in a medium which is already relatively less viscous.

A more detailed analysis of this highly effective ciliary apparatus is actually in progress. The study of its structure and of its development in the embryos of different decapod cephalopods may throw new light on the evolution of the functional relationships between special embryonic structures and protective devices produced by the adult.

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### Alteration in thermal stability of ribosomes from *Drosophila melanogaster* with age

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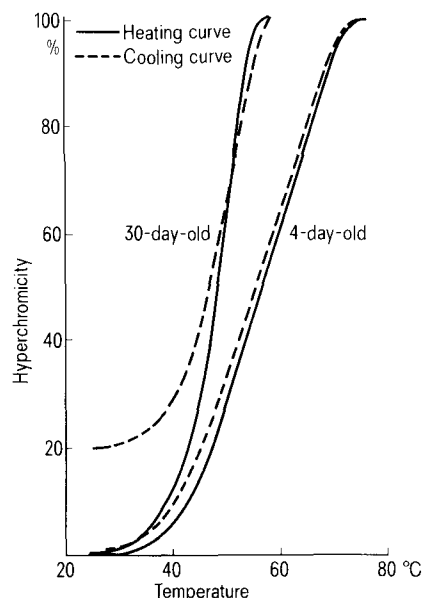
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**Summary.** Thermal analysis of high salt (0.5 M) washed ribosomal monomers from young and old male *Drosophila melanogaster* revealed an 8 °C downshift in the mean temperature of denaturation ( $T_m$ ). Moreover, there was observed a marked loss in the ability of ribosomes extracted from older flies to reassociate upon cooling. These observations suggest that age-dependent alterations in the structural integrity of the rRNA-r protein complex could, at least in part, be responsible for the diminished capacity for protein synthesis in this species with advancing age.

A diminished capacity for protein synthesis with advancing age has been well documented in various mammalian systems<sup>2-7</sup> including man<sup>8</sup>. Similarly, age-dependent decreases in net protein synthesis have been demonstrated in higher insects<sup>9-11</sup>. However, the mechanism(s) underlying this widespread correlate of the aging process have not been fully elucidated. Much of the current research in this area has been directed toward possible age-dependent alterations in the various regulatory factors associated with the ribosome<sup>12,13</sup>. And although the ribosome has been suggested as a possible site where changes could effect the quantity and/or fidelity of protein synthesis<sup>14-17</sup>, little experimental information is available as to what changes occur within the ribosome with age. A rather general finding with advancing age is a decrease in the amount of polysomes with a concomitant increase in 80S monomers<sup>18,19</sup>. These observations could be a reflection of any number of alterations including decreased availability of mRNA, quantitative and/or qualitative changes in the ribosomes, or changes in levels of fidelity of the various accessory factors necessary for the initiation of protein synthesis. Previous studies from this laboratory have demonstrated a 23% reduction in the amount of extractable ribosomes from older *Drosophila* males as well as an increase in the amount of protein dissociated from the high salt washed ribosomes in the presence of increasing concentrations of KCl, some 5-fold at 2.0 M KCl<sup>20</sup>. The current studies were undertaken in an attempt to further characterize and identify the molecular lesions(s) responsible for the previously observed physicochemical alterations in ribosomal structure with advancing age.

**Materials and methods.** Ribosomes from young (4-day) and old (30-day) male *D. melanogaster* (Sevelen strain) were isolated as previously described<sup>20,21</sup>. Males of this strain demonstrate 50% and 90% mortalities of 29 and 40 days respectively, when reared and maintained as previously described<sup>22</sup>. Only ribosomal preparations with an OD<sub>260</sub>/OD<sub>280</sub> ratio between 1.70 and 1.90 were employed for thermal analysis. Melting profiles were obtained on a Beckman Acta CIII UV spectrophotometer equipped with

a Braun Melsungen Thermomix 1480 and Thermograd 1491 regulated temperature bath. The slope-time rise of the temperature as monitored by a platinum thermo-electrode was maintained at 1 °C/min over the operating temperature range of 25-85 °C. The ribosomal monomers were melted in 1 ml Teflon stoppered cuvettes in degassed, deionized DD H<sub>2</sub>O at an initial concentration of 0.5 OD<sub>260</sub>/ml. When no further increase in hyperchromicity was observed for



Thermal analysis of high salt washed (0.5 M) ribosomes extracted from 4-day- and 30-day-old male *D. melanogaster*. Only ribosomal preparations with OD<sub>260</sub>/OD<sub>280</sub> ratios between 1.8 and 1.9 were employed in these studies. The graph above represents the mean change in hyperchromicity at 260 nm/°C of melts obtained on at least 6 separate preparations for each age. Thermal analysis of ribosomes was carried out in degassed, deionized DD H<sub>2</sub>O at a rate of 1 °C/min in jacketed Teflon stoppered cuvettes.