Parabiosis Between Grasshopper Embryos (*Schistocerca gregaria* Förskal; Acridoidea, Insecta)

Parabiosis on insects has frequently been carried out in postembryonic stages, chiefly to study the localisation and functions of the hormonal centres[1-3].

There are no previous reports, to our knowledge, of parabiosis between insect embryos. This perhaps is due to the fact that the eggs have a thick shell and parabionts can only be obtained and made to grow provided an adeguate in vitro explantation technique of the embryos has been achieved. In recent years, several research workers[4-10] have succeeded in obtaining the in vitro development of insect embryos.

By modifying MUELLER's[5] technique, we successfully explanted in vitro and reared parabionts almost up to the stage of the hatching.

Material and methods. Eggs of *Schistocerca gregaria*, bred and reared in our Institute, were used, taking them out at the required developmental stage from those undergoing normal development in sand-filled tubes.

The embryos, at the same developmental stage, were removed from their shells in a sterile Hoyle saline solution. Under a dissecting microscope, the embryos, completely immersed in the saline solution, were cut by means of very fine scissors from the head to the terminal segments of the abdomen along the dorsal midline. 2 embryos are then placed back to back, the edges of the incisions of both embryos were again cut, this time jointly, in such a way as to weld them together.

The parabionts were placed in a few drops of saline solution on the cover of a Petri dish which was then turned upside down on the container so that the embryos could develop in a hanging drop, according to MUELLER's technique[5].

The parabionts develop even when placed on the bottom of the Petri dish, if adequately covered by the saline solution. The Petri dishes, so prepared, were placed in hermetically sealed plastic containers equipped with 2 taps through which they could be oxygenated every 24 h. The containers were then kept in a thermostat at 30 °C. The saline solution was changed every 3 days.

A more efficient development of the parabionts was obtained by explantation in Petri dishes as before but using the same chorion from which they had been extracted as a support.

For histological examination the parabionts were fixed in alcoholic Bouin's fluid, and paraffin embedded. Sections 7 μm thick were then stained with hemalum and eosin.

Results. Back-to-back parabiosis was achieved between embryos at various stages, but only after they had at least completed blastokinesis, at about the stage 22 according the developmental stages established by MIC-

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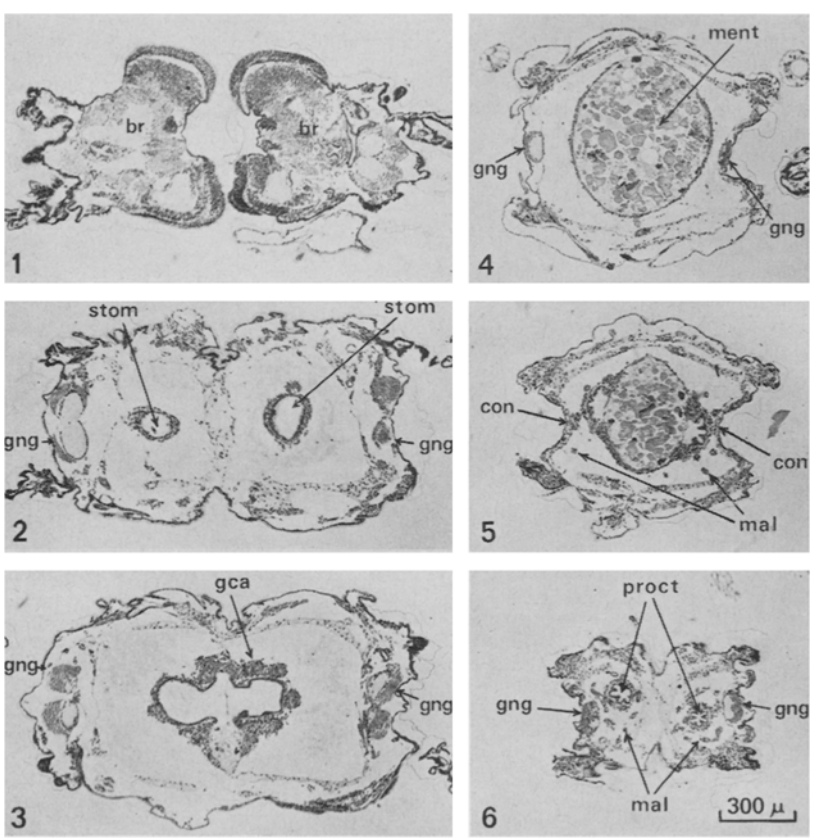

Cross-sections of parabionts explanted in vitro for 8 days from stage 22–23. Lateral sides of sections correspond to the ventral sides of each embryo, the median lines are the fusion's lines. Section at the level of: 1. the head; 2. of the stomodea; 3. of the terminal part of the stomodea; 4. of the first adbominal segments with mesenteron; 5. of more caudal abdominal segments at the end of mesenteron; 6. of the last abdominal segments with the proctodea. br, brain; stom, stomodaeum; gca, gastric caeca; proc, proctodaeum; ment, mesenteron; mal, malpighian tubules; g ng, ganglion of the ventral nerve cord; con, connectives of the ventral nerve cord.

CIARELLI[11], at which period the embryo occupies less than half the shell. The embryos thus sealed together begin to grow in length and in some cases succeed in almost completely engulfing the yolk; development and differentation proceed regularly up to the stage just prior to hatching.

The integument of one individual is in continuity with that of its partner; in most cases between the integument and the mesenteron there is a continuity of the hemocoele and also of the mesodermic derivates, mostly represented by fat bodies. The 2 ganglia chains, which are fully differentiated in their nerve sinuses, as well as the musculature and the fat bodies of the ventral zone of the 2 embryos, are complete and duplicated, as could be foreseeen in back-to-back parabiosis.

No brain fusion was observed (Figure 1), which may be accounted for by the fact that at the time of operation the cephalic regions of both embryos are widely separated by yolk and only draw near, almost contacting one another, after yolk engulfment. By this time differentation of the brain and of the compound eyes has already taken place.

Stomodea and proctodea (Figures 2 and 6) are always duplicated, 1 for each embryo. The individuality of the 2 stomodea persists up to the initial stage of the formation of the ceca rudiments (Figure 3). At this level the stomodeal tubes of both embryos straighten out, each one forming a single cellular lamina adhering to the yolk mass. The edges of both laminae weld together; thus a single mid-gut (Figure 4) is formed out of fusion of the intestinal walls of the 2 embryos.

The proctodea of both parabionts also remain double up to the levels where the malpighian tubules are formed. At this level the proctodea stretch out into a cellular sheet (Figure 5) which comes to lean against the yolk. The lateral, or better, dorsal edges then seal together to form a single tube encasing the yolk. The yolk of both embryos is thus fused into a single mass surrounded, from the stomodeal to the proctodeal region, by a single ento-mesodermic wall derived from the 2 embryos.

In most cases both the heart and mesodermic rudiments of the dorsal region are lacking, because nearly always, in the course of parabiotic procedures, these rudiments are cut away. In the majority of cases the gonad primordia are retained and show up distinctly in the parabionts.

The gonads of the 2 embryos come closer together and, in cases where the gonads are of different sex, the histological features exhibited by the testis rudiment make it resemble an ovary. To clarify this point, further experiments are in progress.

We wished these initial results to be known because the technique of parabiosis between grasshopper embryos could be a useful tool in tackling various embryological and physiological problems concerning the development of insect embryos[12].

Riassunto. Essendo stata messa a punto una tecnica di espianto in vitro di embrioni di *Schistocerca gregaria* si è riusciti ad ottenere lo sviluppo fino a quasi la schiusa di parabionti uniti dorso-dorso a stadi subito dopo la blastocinesi. Si è osservata saldatura e continuità dell'epidermide. Gli abbozzi delle parti ventrali dei parabionti si sviluppano e differenziano in maniera autonoma; solo l'intestino medio risulta unico.

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Cytokinin-Like Activity in Sea Water from the *Fucus-Ascophyllum* Zone

Naturally occurring cytokinins have been extracted from unicellular algae[1], red algae[2] and brown algae[2,3]. The present report deals with the presence of cytokinin-like activity in sea water surrounding the littoral algae in the *Fucus-Ascophyllum* zone. Sea water from this zone is known to support growth of several algae in culture much better than sea water off-shore[4,5].

Sea water was collected during April, May and October from the Swedish west coast, filtered and deep frozen until used. The pH of the sea water was adjusted to 7.5–8.0 and 900 ml of this sea water was extracted with 150 ml ethyl acetate for 15–20 h at 130°C in an apparatus constructed for liquid-liquid extraction of pressed apple-juices[6]. The ethyl acetate was redistilled prior to use. After removing the ethyl acetate in a rotary evaporator, the residue was dissolved in a small volume of absolute ethanol and re-evaporated. This dry residue was stored in a desiccator until further use.

For the cytokinin assay callus cultures isolated from stem segments of *Nicotiana tabacum* var. Alida were used. The fresh weight of each piece of inoculum was about 10 mg. Erlenmeyer flasks (100 ml) were used with 50 ml of agar medium[7] with 3 pieces of inoculum in each. The cultures were incubated for 5 weeks at 30°C in diffuse light from 2 fluorescent tubes (Osram Fluoralux 40 W/77). At the end of the incubation period, fresh and dry weights of the callus tissues were determined. In addition shoots and budlike projections were counted and examined microscopically. The medium contained 3×10^{-6} *M* indole-3-acetic acid, and the sea-water extract was substituted for a cytokinin except in 1 control group, where 5×10^{-7} *M* 6-(methyl-2-buten-1-ylamino)-purin (2 iP[8])

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