Information Transfer in Molluscan Embryos

FLICKINGER[1] first pointed out the possible use of actinomycin to find out whether embryonic information transfer is sequential. According to him *m*RNA for particular organs is "released in the cytoplasm immediately before differentiation". If this view is correct, it should be possible to suppress the differentiation of organs selectively by treating the embryos (with inhibitors like actinomycin) at specific stages. Ideally, it should be necessary to follow up such experiments with autoradiographic and biochemical tests for suppression of RNA synthesis. Such selective organ suppressions, however, would be interesting *per se* and hardly any good examples are known. I have reported[2] the inhibition of pigmented sensorial organs of *Ciona* by actinomycin and chromomycin. The latter drug, known to inhibit synthesis of RNA[3], also binds to DNA, like actinomycin[4,5], and its effects on *Ciona* are similar to those of actinomycin[6,7].

Similar experiments have now been carried out with *Limnaea*. The eggs were collected from leaves of aquatic plants in the pond or in earthen vessels kept in the laboratory.

Effects of 50 γ/ml. of actinomycin and chromomycin A_3 were similar. Short treatments (2 and 4 h) during early cleavage, from the two cell stage onwards, cannot stop organogenesis or even hatching. Prolonged treatment (26 h, 48 h or more) during the trochophore stage exerts a marked effect. Treated embryos do not develop much

beyond the trochophore stage; abnormal trochophores continue to rotate within the egg capsule while controls hatch. Such prolonged treatments at late trochophore or early veliger stages cannot stop the principal organogenesis although abnormalities are induced (suggesting that the drugs have penetrated). Trochophores and veligers at different stages were treated with (100 γ/ml.) actinomycin for shorter periods (90 min). The results confirm that the drug is a very effective inhibitor of morphogenesis at the early stage of the trochophore. Short treatments (with 100 γ/ml.) produce results in the early trochophore which are comparable with those mentioned above, while organogenesis cannot be suppressed at later stages; though malformations and abnormalities do occur. A possible explanation of these facts is that during the trochophore stage a large amount of information is released. Furthermore, treatments at successive stages indicate a "wave like" or rhythmic sensitivity to the toxic effect of drug, that is the embryos die (after organogenesis) if treated at certain later stages. Such successive treatments did not selectively suppress any specific organ but the posterior parts were always more sensitive and in a number of cases practically naked snails (with very rudimentary shells) were seen to hatch. This can also take place if treatment is given at the stages of gastrulation, late morula or early veliger. Sherbet and Lakshmi[8] obtained similar results with *Planorbis exustus*, treated with certain inhibitors of protein and RNA. Similar results, however, have been reported also with lithium chloride[9]. But at certain stages lithium chloride produces marked cephalic abnormalities[9,10] while at no stage of development does treatment with actinomycin (50 γ/ml.) exert such effects.

Unlike sea-urchin[11,12] and *Ciona*[4,7], *Limnaea* eggs treated at the stage of early cleavage with actinomycin (50 γ/ml.) and chromomycin (50 γ/ml.) and then put back into water develop more or less normally and can hatch, although at the trochophore stage treatments of the same duration (4–5 h) or less (2 h) induce abnormalities and prevent hatching. Perhaps binding to DNA occurs more easily at later stages. The marked effects of treatment with 100 γ/ml. of actinomycin show that the eggs are permeable to the drug at early cleavage.

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Addendum: (1) Selective suppression of the "skeleton" in sea-urchin by actinomycin has now been achieved (personal communication from G. Grudice). (2) The problem of permeability of actinomycin in eggs of *Limnaea* at an early stage can be overcome, in principle, by employing labelled actinomycin.

Note added in proof. Incorporation of phosphorus-32 shows indeed a peak at trochophore which gradually declines in the veliger stage. Actinomycin (100γ/ml.) can suppress incorporation by 50μ per cent.

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