

cells but also the eye lumen was entirely free from cellular debris.

**Discussion.** The present study showed intensified and extended pycnotic zones in optic primordia after exposure on day 9 compared to the suppressed cell death following treatment on day 8<sup>2</sup>. The extreme rise in the radiosensitivity of the more advanced stage may be attributed to the differentiation of the radioresistant neuroectoderm cells (day 8) into highly susceptible neuroblasts. Furthermore, the analysis showed that the recovery process does not comprehend all parts of the eye primordium simultaneously. The areas containing only a slight amount of endogenous cell death during normogenesis (dorsal ventricle, lens placode) apparently regenerate faster than the regions with an intense morphogenetic activity (eye stalk, ventral and distal optic vesicle). Moreover, the decrease in the incidence of extensive and massive necrosis (classes 5 and 6) from 65 to 2% within only 9 h demonstrates the restitution efficiency of the early developing eye. However, in view of the large cellular deficiency the massive destruction of the exposed tissue 3 h p.r. may lead to serious long term effects. It has been emphasized<sup>6-8</sup> that despite the high regenerative potency, cell death induced exogenously always has negative consequences for the embryo. Hence the question arises whether the proliferative capacity of surviving cells will be sufficient to restore conditions corresponding to those of the control groups. One way to approach this problem would be to ascertain the total cell number in affected eyes after the completed differentiation. Finally,

besides cell killing one can assume only a sublethal damage of cells (e.g. after irradiation with low doses) which in spite of the absence of necrosis produces developmental disorders<sup>9</sup>.

The particular complexity of the necrosis pattern during early mammalian organogenesis<sup>10</sup> implies that further studies are necessary to clarify the still obscure role of cell death in the teratogenic pathways.

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## Cyclic nucleotides phosphodiesterase activity changes in early chick development

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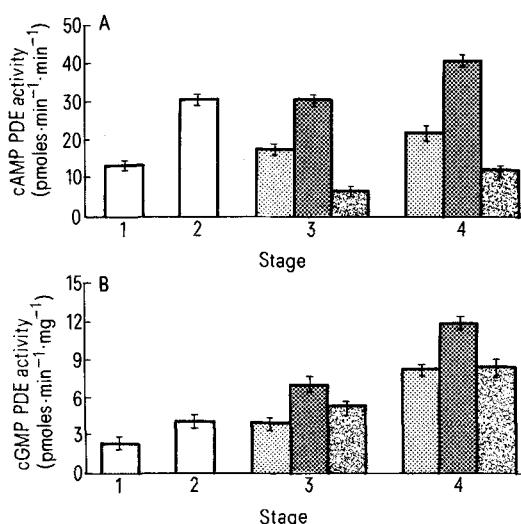
**Summary.** Differences in the activity of cyclic nucleotides phosphodiesterase develop in different germ layers during the gastrulation of the chick embryo.

In recent years it has been shown that the addition of external cAMP phosphodiesterase (PDE) disturbs the gastrulation process<sup>2</sup> and that reaggregation of chick embryo cells is cAMP-dependent<sup>3</sup>. Also, it has been assumed that the direction of the primitive streak elongation in chick embryo depends on cyclic AMP (cAMP)<sup>2,4</sup>.

In this paper the results of a study of the changes in cAMP and cyclic GMP (cGMP) phosphodiesterase (EC 3.1.4.17) activities in the different germ layers of the early chick embryo are reported.

**Materials and methods.** 8-<sup>3</sup>H-cyclic-AMP, 8-<sup>3</sup>H-cyclic-GMP were purchased from Amersham, England; cyclic-AMP, cyclic-GMP, 5'-AMP, 5'-GMP from 'Reanal' (Hungary), TLC plates 'Silufol' from 'Kavalier' (Czechoslovakia). Fertile White Leghorn eggs were incubated at 38.5 °C to obtain embryos at stages 1-4 of development<sup>5</sup>.

For PDE activity measurements the following areas of embryos were used: at stages 1 and 2 - all area pellucida, at stages 3 and 4 - primitive streak, epiblast and meso- and endoblast together. Embryos were dissected with tungsten needles and separated parts of embryos were rinsed in Dulbecco-Fogt physiological saline, collected in the homogenization buffer containing 40 mM TrisHCl, pH 7.8, 5 mM MgCl<sub>2</sub> and homogenized in a Dounce microhomogenizer ('Kontes'). cAMP and cGMP PDE activities were



Changes of cAMP phosphodiesterase (A) and cGMP phosphodiesterase (B) activities (pmoles · min<sup>-1</sup> · mg of protein<sup>-1</sup>) during early chick development from stage 1 to 4 (□, area pellucida; ▨, primitive streak; ▩, epiblast; ▤, meso- and endoblast). The activities are expressed as means ± SD for 10 separate experiments.

measured in the crude extracts in homogenization buffer, final volume 50  $\mu$ l, containing 20  $\mu$ M  $8\text{-}^3\text{H-cAMP}$  or 20  $\mu$ M  $8\text{-}^3\text{H-cGMP}$  respectively. The samples were incubated at 37°C for 15 min and the reaction was stopped by the addition of 30  $\mu$ l of 10% TCA. cAMP and 5'-AMP or cGMP and 5'-GMP were separated on 'Silufol 254' TLC plates, using a solvent containing isopropylalcohol, 25% water solution of  $\text{NH}_4\text{OH}$  and water (7:1:2)<sup>6</sup>. The nucleotides were detected under UV-light, the spots were scraped off and radioactivity (dpm) counted in toluol scintillation fluid using a liquid scintillation counter ('Rackbeta', LKB). Protein was determined by the method of Lowry et al.<sup>7</sup>.

**Results.** The data obtained demonstrate a remarkable, more than 2-fold increase on the cAMP PDE activity in the development of the embryo from stage 1 to stage 2 (fig. A). During the gastrulation (stages 3 and 4 embryos) the cAMP PDE activity in the epiblast remained practically constant at the level reached in the stage 2 embryo, whereas the enzyme activity decreased in the primitive streak and in the meso- and endoblast. In the latter cells, the level of the cAMP PDE activity was about 4 times lower than in the epiblast. In fact it means that a gradient of cAMP PDE activity (epiblast  $\rightarrow$  primitive streak  $\rightarrow$  meso- and endoblast) develops in the embryo during gastrulation.

As shown in figure B the cGMP PDE activity increased steadily during the development of the chick embryos from stage 1 to stage 4. The differences in the cGMP PDE activity between the different germ layers were smaller in comparison to those of the cAMP PDE. Unlike the cAMP PDE activity, no clearcut gradient of cGMP PDE activity was formed in the embryo during gastrulation.

**Discussion.** Taking into account that cells move stepwise during the primitive streak elongation<sup>8</sup>, and also the suggestion that cAMP acts as an attractor in primitive streak elongation<sup>2,4</sup>, it could be assumed that the mechanism underlying the process of gastrulation of the chick embryo is similar to that functioning in the slime mold *Dictyostelium discoideum* development<sup>9</sup>. In slime mold development starvation leads to the formation of the center of aggregation which emits waves of cAMP, and the aggregation of the cells occurs in the field of the propagating waves, the cells moving by jumps. In the case of the chick embryo the 'center of aggregation' could be located in (or near) Hensen's node which attracts the cells of the epiblast, thus

forcing them to move 'downward' through the Hensen node.

In the case of *Dictyostelium*, high activity of cAMP PDE is necessary for the decrease of the concentration of cAMP during the propagation of the waves of the attractor. Higher activity of cAMP PDE in the epiblast in comparison to that in mesoblast and endoblast is in agreement with the assumption made above.

The decrease of cAMP PDE (and also cGMP PDE) activity in the primitive streak could be related to the increase of catabolic activities in this area. Karner and Leikola<sup>10</sup> showed that the activity of the lysosome enzyme acid phosphatase increases in the primitive streak. Also it has been shown in our laboratory that the RNA and protein synthetic processes which could compensate the loss of the cellular material are less intensive in the meso- and endoblast in comparison to those in the epiblast (unpublished results).

**Conclusion.** Formation of a gradient of cAMP PDE activity in chick embryo during gastrulation was observed. The gradient observed could be considered as an additional evidence supporting the hypothesis according to which cAMP participates in the morphogenesis of the chick embryo.

- 1 I should like to thank Dr Raivo Vilu for helpful discussion during the course of the work.
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## Inheritance, maternal influence and biochemical analysis of an egg color polymorphism in *Ophryotrocha diadema*\*

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**Summary.** In the Polychaete worm *Ophryotrocha diadema*, the yellow coloration of egg yolk is due to the selective uptake of lutein from food. The genetic control of this mechanism depends on a single locus with 2 alleles, the dominant Y (yellow) allele, and the recessive y (absence of coloration) allele.

Natural populations of most species of the Polychaete genus *Ophryotrocha* are remarkably uniform with regard to externally visible characteristics. Until now, only a transpecific polymorphism regarding a yellow or white egg coloration has been reported by Akesson<sup>1</sup> in *O. diadema* and by Sella<sup>2</sup> in a population of *O. puerilis siberti* from Roscoff. In *O. diadema*, as in *O. p. siberti*, this polymorphism consists of the presence or absence of yellow pigmentation in both eggs and the animals' body walls. When animals are fed with spinach or nettle, the difference between the 2 forms is very clear. Pigment accumulates first in cells of the gut wall and later in nurse cells and oocytes.

**Genetic analysis of the polymorphism.** Crosses were performed with animals from white and yellow egg stocks, obtained through the courtesy of Prof. Akesson. Animals were reared at constant temperature (20°C) in vessels containing 10 ml of filtered sea water with density of 0.024 g/l and were fed with parboiled spinach. Food was always in excess and the sea water was changed once a week. Because *O. diadema* is a contemporary hermaphrodite, single mating pairs were made only when both mates showed oocytes, in order to be sure of their phenotype. F<sub>1</sub> progeny of crosses of yellow with white egg individuals always produced yellow egg individuals. Results of 18 mat-