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Early chick embryo genesis

Introduction

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The problem of differentiation is the most important and mysterious of those studied by embryologists. It arises when studying primitive development as well as the genesis of each system or organ, especially in embryos with high regulation potency during early stages. Cases in point are avian or mammalian eggs, the human egg included. Initially they have no other determination than that of axes (cephalo-caudal, dorso-ventral) and no territorial determination. For obvious reasons the bird egg is an ideal material for experiments on amniotes. It serves as a model for the explanation of normal or pathologic development in higher vertebrates. What do we know of the causes of differentiation? Although it is, today, feasible to elicit certain types of differentiation (polyembryony, neuralisation, skeletal, digestive, liver structures, various malformations, etc. ...) it is usually impossible to appreciate the underlying mechanisms. The papers collected in this issue attempt to explain some differentiation processes with the aid of modern techniques.

One chapter deals with the formation of new molecules during differentiation. Are the sulfated proteoglycans synthesized by the definitive chondroblasts that form vertebral cartilage identical to those in other organs or in young chondroblasts? Holtzer et al. show that they are specific substances elaborated when the neural tube or the notochord have exerted their inductive action on somitic mesoderm. It is interesting to confirm at the molecular level results previously obtained by classical morphology experiments. What still remains to be found is the mode of action and the nature of the inducer of cartilage and whether it is the same for all cartilaginous organs. Several hypotheses have been proposed by different authors. None has yet received experimental confirmation.

H. Lee and G. W. Kalmus have studied the teratogenic effect of BrdU (5-bromodeoxyuridine) on young embryos explanted in vitro. This thymidine analog induces different malformations, depending on the stage at which it is administered: first heart anomalies, thereafter deficient closure of the posterior brain, then of the intermediate or anterior brain and finally somite abnormalities. These differential sensitivity periods overlap widely, but differences with controls are statistically significant. According to Lee and Kalmus, these differences arise from an inhibition of the synthesis of some specific messenger RNAs. This interpretation is put forward as a hypothesis.

Françoise Dieterlen-Lièvre has investigated the important problem of the origin of blood cells in the young chick embryo. It should be recalled that, at 48 h of incubation, the circulation is very active and red cells are already functional oxygen transporters. Using the nuclear markers described by N. Le Douarin in the quail, F. Dieterlen demonstrates, by means of a grafting technique that results in chick/quail chimaeras, that, although the first erythroblasts derive from the yolk sac, other stem cells subsequently form in the embryos and mingle with those originating from the yolk sac. At an early stage, they colonize organs like the thymus, the bursa of Fabricius or the spleen, which are relays in haemopoiesis.

Thus, as often happens in development, one system progressively gives way to another. This is evocative of the differentiation of other embryonic systems, such as the nephroi, the vascular apparatus, etc. ...

P. E. Messier, on the strength of experiments and electron microscope observations, attempts to explain the invagination processes in terms of intracellular modifications as Holtfreter did earlier for amphibian embryos. He thinks that neural plate invagination results from shifting of nuclei towards the basal region of cells. These movements would be coordinated by microtubules or microfilaments. These conclusions are based on experiments involving the combined action of substances, such as formamide, cytochalasin and of physical factors, such as low temperature which modify microtubule configuration and consequently nuclear topography. It is possible that neighbouring cells also play a part in the formation of the neural groove. The author stresses the hypothetical nature of his explanation.

Thus recent researches on the chick embryo are directed towards a deeper analysis of the differentiation processes. Biochemical, immunochemical and electron microscopic techniques are called on in an attempt to explain induction or invagination processes, positioning of migrating cells, and developmental abnormalities. The studies presented in this issue, apart from that concerning the origin of blood cells, lead to explanations which are yet only hypotheses and which call for further experiments. Science progresses by small steps and major problems are still to be solved.

The differential susceptibility of early chick embryos to 5-bromodeoxyuridine*

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The thymidine analogue, 5-bromodeoxyuridine (BrdU), has been shown to inhibit the synthesis of cell specific macromolecules without interfering markedly with cell growth and multiplication[1–10]. Since BrdU is readily incorporated into DNA synthesized in vitro and into DNA of both prokaryotic and eukaryotic cells, it has been assumed that its action is DNA-linked[10,11]. Teratogenicity of BrdU has been reported in sea urchin[12,13], amphibian[13], mouse[14,15], rat[16], hamster[17], and chick embryos[18–20]. This paper is concerned primarily with the effects of BrdU in explanted early chick embryos at different stages of development.

Effects of BrdU on early chick embryos

Explanted chick embryos at stages 3–7[21] were cultured[22] on nutrient medium (thin albumen) containing different concentrations of BrdU until the majority of corresponding controls had reached stages 9–11. 1 μg/ml caused a growth retardation of stage 4 or younger embryos, but had no apparent effect on older embryos. Irrespective of stage at explantation progressively larger doses of BrdU resulted in higher persentages of disturbed development. Of all the concentrations of BrdU tested, 8–10 μg/ml at stages 3–5 and 10–14 μg/ml at stages 6–7 appeared appropriate for examination of teratologic effects because of elevated percentages of surviving embryos with one or more discernable abnormalities (figures 1–3).

BrdU, at teratologic doses, inhibited segmentation of axial mesoderm regardless of stage at treatment. The blastodermal expansion and erythropoiesis were usually unaffected. Heart development was significantly affected only in those treated at stage 3. The magnitude of inhibitory action of BrdU in brain development was clearly stage-dependent: at stage 4 or earlier, it resulted in an open brain region (figure 1); if applied at stage 5, it inhibited the closure of the midbrain and, to some extent, the hindbrain, but the forebrain closure was unaffected (figure 2). However, BrdU had no apparent effect on brain roof closure of embryos treated at stages 6–7 (figure 3). BrdU-insensitive processes are not uncommon in developing systems, e.g., echinochrome synthesis by sea urchin embryos[12], haemoglobin synthesis by erythrocytes[2], chondroitin sulphate synthesis by chondrocytes[4], etc. Weintraub et al.[5] suggested that normal differentiation involves the institution of a programme that is resistant to BrdU. It does not, however, explain the reason why BrdU selectively inhibits the differentiation of certain tissues and/or cell functions. The observed brain defects were not due to impairment of inductive interactions between chordamesoderm (Hensen's node) and competent epiblast[19]. Microscopic studies revealed that neural tissue was most susceptible to BrdU treatment. Neuroepithelial cells often had an enlarged nucleus, a characteristic of undifferentiated cells[19]. Pycnosis and chromosomal abnormalities were also observed. Mitotic figures were

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