The use of intracellular horseradish peroxidase (HRP) injection for cell lineage studies in early mammalian development.

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Embryonic cell lineages may, on principle, be studied using any of the following markers: (1) intrinsic morphological markers (like characteristic organelles, pigment granules, etc.); (2) intrinsic biochemical characteristics (including e.g. enzymes, polypeptide patterns, antigens); (3) exogenous tracers (vital dyes, carbon particles, enzyme tracers like HRP, or radioactive precursors); (4) genetically determined phenotypic differences as in mosaics and experimental chimaeras. In (2) to (4), histochemical methods are very helpful to reveal spatial cell arrangement patterns.
Since early mammalian embryos lack obvious cytological markers which would allow the morphological identification of various cell types and their precursors, theories on cell lineage remained very controversial as long as conclusions were based primarily on morphological observations. Cell transplantation studies performed in recent years using approach (4) have considerably added to the available information, and detailed fate maps have been proposed. However, the impressive regulative capacities of early mammalian embryos leave some doubts as to the validity of the derived cell lineage patterns when based exclusively on transplantation experiments (for discussion, see DENKER, 1983). This may be one of the reasons why there are still 3 conflicting hypotheses on the mechanism of the first process of determination in mammalian development, i.e. the formation of trophoblast and embryoblast (inner cell mass, ICM): the so-called "segregation hypothesis", the "inside-outside hypothesis", and the "polarisation hypothesis", putting emphasis on either intracellular determinants or on cell-cell interactions.
In order to study cell lineage without disturbing normal spatial cell arrangements, we are using intracellular HRP injection (for details of the procedure, see BALAKIER & PEDERSEN, 1982). The second cleavage division of the mammalian egg is typically asynchronous, so that 3-cell stages are consistently seen. It has been proposed that the first cell to divide contributes more descendents to the ICM than the other one (see BALAKIER & PEDERSEN, for literature). We are injecting HRP iontophoretically into either (a) the non-divided (late-dividing) blastomere or (b) the descendents of the earlier dividing blastomere (still connected by a cytoplasmic bridge). Development of the embryo is then allowed to proceed to the blastocyst stage, when the histochemical HRP reaction is performed and patterns are studied on serial sections of plastic-embedded material. Advantages and problems connected with this type of approach will be discussed.

References

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XXVI. SYMPOSION

DER GESELLSCHAFT FÜR HISTOCHEMIE

A B S T R A C T S

(In alphabetical order)

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